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LIFE HISTORY OF PELTANDRA VIRGINICA

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(WITH FORTY-NINE FIGURES)

Introduction

A morphological study of *Peltandra virginica* Kunth was made to assemble data which would give a rather complete life history of a widespread plant and a basis for comparison within and outside the Araceae. Features neglected or incompletely ascertained in the plant and the family as a whole were studied as fully as possible.

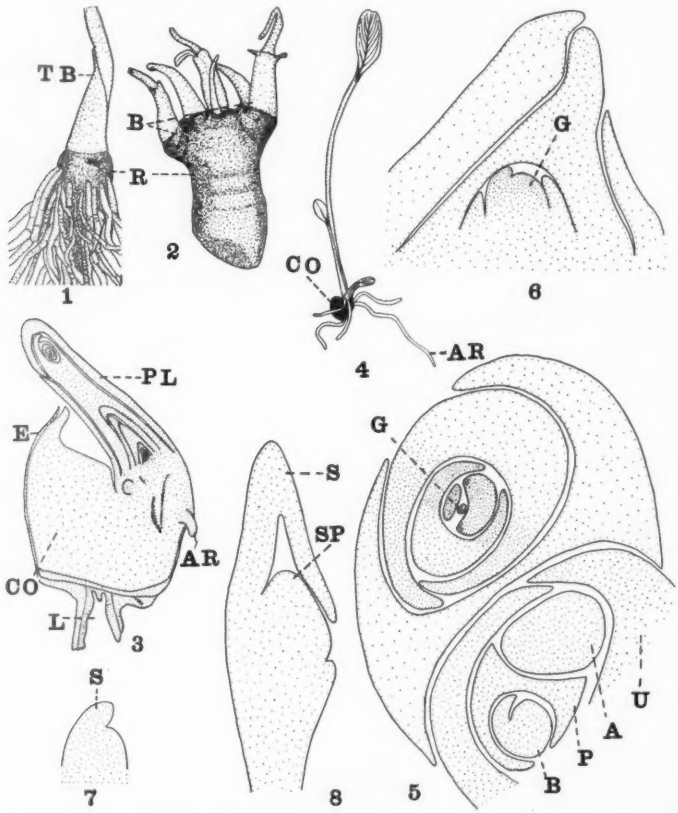
Comparative studies on the aroids (**13, 23**) have been of interest since ENGLER (**10, 11, 12**) pointed out that in spite of appreciable variation there were unifying tendencies in the group. The only detailed morphological account of *Peltandra* (**7**) deals with part of the development of the pollen. Additional reports include a microchemical study of the seed and its germination (**18**), a demonstration that the seed can germinate in almost total absence of oxygen (**8**), and an account of seed frequencies (**9**).

Observations

Peltandra is found throughout the eastern United States and has been reported west of the Mississippi River. It is an inhabitant of fresh water marshes, especially along the banks of tidal rivers. The stem is a subterranean vertical rhizome (figs. 1, 2*R*), cylindrical, and with a diameter in mature, unbranched specimens of 8 cm. The leaves are so compactly arranged that internodes appear nonexistent. The rhizome sometimes branches (fig. 2*B*) and the branches become separated from the parent plant as the basal portion of the rhizome decays. Since branches as well as parent axis form erect rhizomes, there results characteristically a cluster of plants.

Some rhizomes bear 100 or more adventive roots, which reach a diameter of

about 7 mm. in their proximal portions. Roots arise acropetally on the rhizome (fig. 1) and sometimes pierce the sheathing leaf bases. Wrinkling of the outer cortical tissues of roots of seedlings and older plants occurs, sometimes contracting to almost 50 per cent of the original length in some regions.



FIGS. 1–8.—Fig. 1, plant collected in October. Fig. 2, branched plant in dormant state with roots removed. Fig. 3, longisection of germinating seed (*CO*, cotyledon; *L*, lumen of haustorial cell). Fig. 4, seedling 16 days old with cotyledon (*CO*) still within pericarp. Figs. 5, 6, transverse and longisections through apex of mature rhizome. Figs. 7, 8, longisections of young inflorescences.

Above the concave top of the rhizome during most of the year is a large conical terminal bud (fig. 1*TB*) about 15 cm. long, extending from the top of the rhizome to the soil surface. From this bud, containing rudiments 15 cm. to 50 μ long, only a part of the outer, older, and larger rudiments emerge in late spring; the remaining ones, excluding those which abort during the interim, emerge during the fol-

lowing year. The various regions of the leaf are clearly defined. Leaves of mature form and size have a sheath 4.5–6.5 dm. long (including the subterranean portion extending from the soil surface to the apex of the rhizome), a stalk 2–4 dm. long, and a sagittate to hastate lamina reaching at times a length of 5 dm. (from the acuminate apex to the rounded basal lobes) and a width of 2 dm.

The larger inflorescences are about 70 cm. long, from the subterranean proximal region of attachment of the peduncle to the distal end of the spathe. The dark green spathe is 20–30 cm. long and extends a few centimeters beyond the inclosed spadix. While there is much variation, most plants in Maryland are at the height of their vegetative and flowering activity by the end of May or early June. After pollination, which occurs at this time, the distal part of the spadix, covered with staminate flowers, disintegrates, together with the distal part of the spathe, marked off by a slight invagination. The erect inflorescences bend below the attachment of the spathe to the peduncle, the fruiting spadix finally pointing toward or resting on the soil. The fruits, surrounded by the enlarged basal part of the spathe, ripen in August or September.

Seed and seedling

The dark green to brown obovoid fruits measure about 1.5 cm. long and 1 cm. wide, and show an apical scar marking the former position of the style. Within the membranous pericarp lies usually one, but sometimes as many as three, seeds imbedded in a colorless jelly-like material.

To the conspicuous, flattened-globular, micropylar portion of the seed is attached an obovoid chalazal appendage about 2–2.5 mm. long, which marks the position of the large haustorial cell of the endosperm. Within the seed lies the large embryonic sporophyte enveloped by the residual endosperm and the almost entirely withered parent sporophytic tissue. The layer of remaining endosperm surrounding the embryo is one cell thick, except in the region nearest the empty haustorial cell, where it is about seven cells thick. The integuments have become brownish and shriveled, but the parent sporophytic tissue in the chalazal region forms a thick coat around the empty haustorial cell.

The pale green cotyledon forms the greater part of the embryo, and has almost the same size and shape as the more conspicuous micropylar portion of the seed. In a deep groove of the cotyledon near the apex of the seed lies the narrowly conical, green plumule, about 9 mm. long and 3.5 mm. in diameter at its base. It contains six or seven foliar structures. Since it is partly encircled by the upgrowth of cotyledonary tissue, it forms with the cotyledon a compact body. The radicle does not form a group of tissues topographically delimited from the surface of the rest of the embryo. Occasionally the hypocotyledonary portion of the radicle appears on the side of the cotyledon as a poorly defined swelling, 1.5 mm. thick and 4–5

mm. wide. About 1.5 mm. below the base of the plumule on its exposed side lies the primary root, a slight rounded protuberance less than 1 mm. in diameter. Around the primary root the surface of the hypocotyl is marked by three to five small, circular, partially hyaline areas which indicate the position of adventive root primordia.

Usually the seeds germinate in April, but in some cases they germinate in the late summer of the year the ovules were fertilized, and while the fruits are still attached to the parent plant. By the time germination occurs the ovary wall is usually so decayed that it presents no mechanical obstacle to germination. As the plumule rises from its cotyledonary groove (fig. 3*PL*), the membranous envelope (*E*), consisting of the remains of endosperm and integuments, is ruptured. Although the primary root generally fails to elongate, it may attain a length of 2.5 cm. before withering. The adventive roots (fig. 3*AR*) grow and elongate rapidly; the first ones are small and short-lived, those formed later approach by degrees the mature size. Figure 4 shows a seedling, with a few adventive roots (*AR*) and the first three cauline foliar structures, 16 days after germination began. The first emergence was a bladeless sheathlike structure about 2 cm. long, the second and third possessed elliptical laminae, and the third alone a distinct stalk below the lamina. The primary axis increases rapidly in diameter, so that the young rhizomes show an obconical tapering.

Mature plant

Branching.—The seedling behaves as a monopodium until initiation of the first inflorescence, which is developed directly from the apex of the primary axis. Apical growth is then carried on by a bud which develops in the axil of the penultimate leaf. In the axil of the ultimate leaf a second bud is formed, which gives rise only to a basal acroscopic, two-keeled prophyll and a second inflorescence at its apex. The bud in the axil of the penultimate leaf completely displaces the original apex of the shoot in prominence and central position, so that outwardly the branching system appears unchanged, and the branch developing from this bud also ceases its apical growth with the formation of an inflorescence, after it has given rise to its share of leaves. Apical growth of the plant is then maintained by a bud in the axil of the penultimate leaf of the branch. The process is continued; and though a mature plant has an erect vertical rhizome, it is actually composed of superposed branches of increasingly higher rank. Engler (10), recognizing the sympodial nature of the shoot in practically all the Araceae, regarded the shoot corresponding to the one arising from the bud in the axil of the penultimate leaf of the *Peltandra* shoot as a unit in the structure of the plant and called it a *Fortsetzungspross* (continuation shoot).

As in the primary axis of the seedling, a second inflorescence (fig. 5*B*), together with a two-keeled prophyll (*P*), is borne in the axil of the ultimate leaf (*U*) of each

continuation shoot. In several hundred plants examined in 5 years, inflorescences were never observed in other positions on the continuation shoot. The second, or lateral, inflorescence originates after the first (fig. 5*A*) and always lags behind it in development. Both inflorescences of a continuation shoot, because of their position in initiation and development, are enveloped by the ultimate leaf.

Frequently the antepenultimate leaf of a continuation shoot bears an axillary bud, the *Vermehrungsspross* (**12**) or vegetative bud (**28**). In a series of plants collected in 1939, the vegetative bud was found on 118 out of 188 continuation shoots. Some plants bear no vegetative buds whatever; others show them only in part; and still others bear one in the axil of every antepenultimate leaf or leaf rudiment. Vegetative buds, if they develop at all, result in the branching of the rhizome and subsequent vegetative multiplication of the plant. In *Symplocarpus* and *Lysichiton* (**28**) the vegetative buds occur in a definite position, that is, in the axil of the lowest leaf of the continuation shoot, but do not develop into branch shoots. In *Peltandra* the buds often do not develop, but many do, some after lying dormant for a time and after the surrounding organs have emerged and disintegrated.

In a series of plants collected from 1937 to 1939, 207 out of 300 continuation shoots showed vegetative buds in the axils of the antepenultimate leaves; two of the 207 also showed them in the axils of the ante-antepenultimate leaf. None were observed in the axils of older leaves, except on the primary axes of seedlings. In one such case thirteen vegetative buds were found, one in the axil of each of thirteen leaves. Vegetative buds were never observed in the axils of the ultimate or penultimate leaves of a shoot.

Morphologically both the vegetative bud and the continuation shoot arise as buds of equal rank, but they differ in appearance and composition as well as in development. Both start with two-keeled, acroscopic prophylls. On the continuation shoot the next foliar structure is a leaf with a lamina of mature form and size; on the vegetative bud one or two more bladeless leaves are borne, while the laminae on subsequent leaves attain only by degrees the mature form and size. The vegetative bud is flattened dorsiventrally much more than the bud of the continuation shoot. After initiation, development of the bud of a continuation shoot is rapid, while that of the vegetative shoot is much slower and sometimes even appears to cease.

NUMBER OF LEAVES PER CONTINUATION SHOOT.—ENGLER pointed out that the number of laminated structures appearing in a growing season, or on a continuation shoot, is often limited in the Araceae, only one being formed in species of *Amorphophallus* and *Philodendron*. In *Peltandra* the number in general decreases with increase in size and with maturity of the plant. On the other hand, branching of the rhizome, through development of vegetative buds, is apparently associated with an increased number of foliar structures on a continuation shoot.

The minimum number of foliage leaves found on a continuation shoot was three.

Once attained, subsequent shoots do not continue bearing this number exclusively, although in one case three foliage leaves were found retained for three consecutive shoots. There was no regularity in the succession of shoots bearing three, four, and five or more foliage leaves in specimens collected over a period of 4 years.

The following diagram is based on ENGLER'S method of summarizing shoot structure types in the Araceae (**10**), and has been modified to represent the complete record of a single specimen at the height of its activity. A continuation shoot and a vegetative bud of equal rank are represented by one horizontal row, and each is connected by a vertical line with the leaf in whose axil it is borne. Lateral inflorescence branches are similarly connected by vertical lines, but placed above their parent axes for clearness. Horizontal lines connect organs at successively higher levels, as read from left to right, on the same shoot.

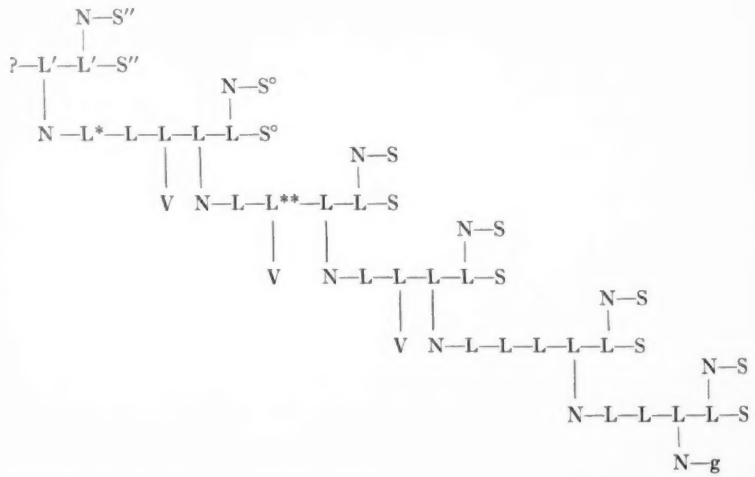


Diagram of mature plant of *P. virginica* collected June 9, 1939, dissected to show number and arrangement of organs, emerged and in rudimentary state. L, leaf; N, two-keeled prophyll; V, vegetative bud (constituent rudiments not indicated); S, spathe and spadix; S″, inflorescence already flowered; S°, inflorescence about to flower; ′, degenerate organs emerged or aborted in 1938; *, first emerged leaf; **, first rudiment of terminal bud (all organs following also exist in bud as rudiments); g, apical growing point of rhizome.

ESTIMATED AGE OF RUDIMENTS AT TIME OF EMERGENCE.—Characteristics of mature plants such as that diagramed may be enumerated as (*a*) small number of leaves (3–5) on a continuation shoot; (*b*) large number of inflorescences for time of year in which examined; (*c*) mature form and large size of leaf (not so reliable an index as the others); (*d*) unbranched rhizome with large diameter; and (*e*) vegetative buds either not initiated or arrested in development after attaining position

for emergence of rudiments. Plants selected by these criteria as mature agreed, to a certain extent, in number of leaf and inflorescence rudiments contained in the terminal bud during the dormant season, in number of organs emerging during the growing season, and in number of primordia added to the bud in summer by activity of growing points of successive continuation shoots. In view of this uniformity, periodic examinations of this type of specimen should reveal, within limits, details of sequence of the emergence of rudiments and their initiation, and the time elapsed from formation to emergence of a leaf or an inflorescence. The numerical range of leaves and inflorescences in mature, rudimentary, and aborted condition

TABLE 1

NUMBER OF LEAVES AND INFLORESCENCES FOUND IN MATURE, RUDIMENTARY, AND ABORTED CONDITION ON MATURE PLANTS THROUGHOUT THE YEAR

Month	Condition of plant	Spadices in bud	Leaves in bud	Spadices in flower or fruit	Emerged leaves	Aborted spadices	Leaves per continuation shoot
Jan.	Dormant	9–10	.....	0	0	0–1	.....
Feb.	Dormant	10	.....	0	0	0–2	4
Apr.	Dormant	10–11	.....	0	0	0–1	.....
May	Dormant	10–12	20–25	0	0	0–2	3–5
June	In flower, fruit, and foliage	6–8 (10)*	15–18	3–5 (6)†	6–8	0–1	3–5
July	In foliage and fruit	7–9	17–21	3–5	5–9	0–1	3–5
Aug.	In foliage and fruit	8–11	21–25	2–4	5–6	0–2 (3)†	3–5
Sept.	Dormant	10–11	ca. 23	0	0	0–2	4–5
Oct.	Dormant	10–12	20–22	0	0	0–2	3–5

*Only one specimen found with ten rudimentary inflorescences at this period; the two oldest seemed about to flower.
†Only case of its kind found.

found on mature plants at various times during the year is shown in table 1. In an average mature specimen, five to nine of the twenty-odd leaves in the terminal bud at the beginning of May, together with three or four of the ten to twelve inflorescences present, emerge by the early part of the summer. Usually one to three leaves and not more than two inflorescence rudiments (the oldest and outermost of the terminal bud) abort between yearly emergences, and are found outside the terminal bud after the yearly crop of organs has emerged and degenerated. Thus about half the rudiments in a terminal bud early in May remain intact in the bud during the subsequent summer and dormant season.

Rudiments initiated during the summer (July–October) are added to those already present in the terminal bud, while the latter increase in size and assume the relative positions occupied by the recently emerged organs before emergence. By October, formation of new rudiments has apparently ceased, since the number re-

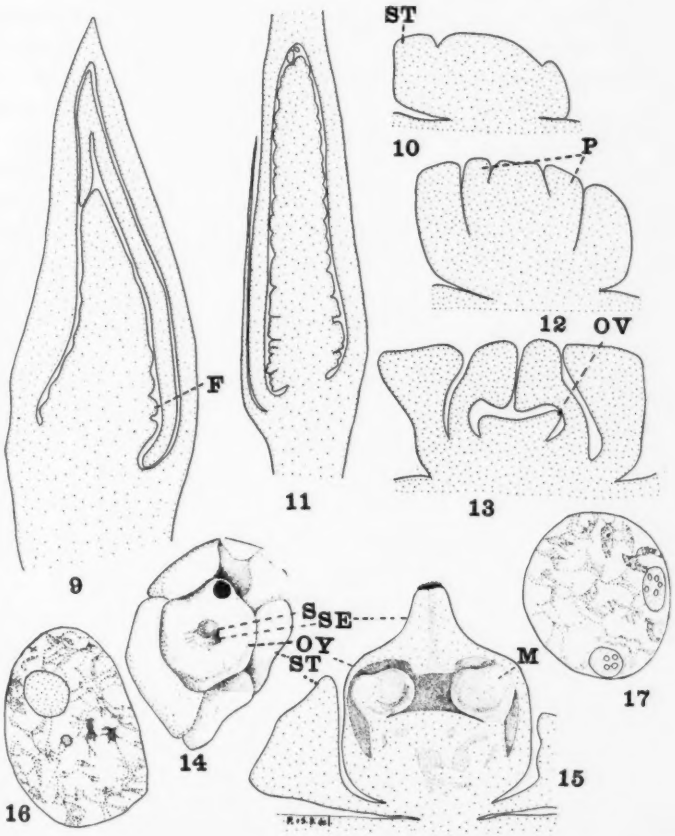
mains the same from October to May and no abortions were observed during this period. It appears that rudiments initiated prior to October of one year do not emerge until about 20 months later (cf. *Symplocarpus* **27**), shortly before the second succeeding summer.

APEX OF SHOOT AND ORGAN INITIATION.—The growing point of the rhizome and the youngest organ primordia lie in a slight apical depression, at the center of which the growing point of the youngest continuation shoot (figs. 5, 6, *G*) can be seen as a dome-shaped structure, overarched and enveloped by leaf primordia. First indications of a leaf are periclinal divisions in the hypodermal layer of the apical region. Very early the tip of the young primordium is marked off by its less meristematic appearance. In the young leaf it is disproportionately large as compared with its relatively insignificant size (about 5 mm.) in the mature organ.

The spathe appears to originate just as a leaf does, although a detailed study of this was not made. Its nature is soon revealed by failure to show the adaxial thickening that characterizes the early stages of petiole-midrib development, by elongation of the axis below its insertion in the formation of the peduncle, and by its position relative to the rapidly developing bud in the axil of the penultimate leaf. If a vegetative bud is to be formed in the axil of the antepenultimate leaf, it appears about the same time as the bud which gives rise to the new continuation shoot. The bud which gives rise to the lateral inflorescence is initiated later. Like the leaf, the spathe early surpasses and envelops the former growing point (figs. 7, 8, *S*) and forms a conspicuous tip, although it develops neither a stalk nor a blade. As the axis below the insertion of the spathe elongates, the former shoot apex develops directly into the spadix (fig. 8*SP*), the outline of which soon becomes slightly undulate. The number of swellings increases acropetally as those more basally situated become pronounced and flattened (fig. 9*F*).

As a result of continued distal flattening, the floral primordia appear in section as inverted truncate wedges. The primordia on the basal part of the spadix give rise to lateral members (fig. 10*ST*), appearing usually as incomplete or infrequently as complete rings, which mark the beginning of the staminodes. Later the primodia give rise to a ringlike lateral growth just within the staminodes (figs. 11, 12*P*). At first the staminodes, the newly formed ring, and the central truncate portion of the primordium develop at about the same rate. Subsequently the ring surpasses the other elements, curving centripetally as it envelops the central region. The edges of the ring, as they grow toward each other, flatten in a direction perpendicular to the long axis of the spadix and form the narrow, slitlike, stylar canal (fig. 13). The proximal part of the former ringlike structure extends centrifugally (fig. 13), forming the ovary; and the central truncate part of the floral primordium becomes the flattened basal placenta which gives rise to the ovules at points around its edge (fig. 13*OV*).

The more apical and by far the larger number of floral primordia do not give rise to lateral members (fig. 11); the flattened protuberances merely increase in bulk. Eventually these primordia, which give rise to the staminate flowers, be-



FIGS. 9–17.—Figs. 9, 11, longisections of young inflorescences. Figs. 10, 12, 13, same of young pistillate flowers. Fig. 14, mature pistillate flower from above. Fig. 15, longisection of same. Figs. 16, 17, early meiotic stages in microsporocyte nuclei.

come roughly rhomboidal to hexagonal when viewed in a section tangential to the surface of the spadix. Undulations arise along the edges of these staminate flowers and mark the origin of individual microsporangia, which usually occur in pairs. As development proceeds, adjacent pairs of microsporangia often become separated by sharp invaginations. This compact structure, bearing usually

sixteen to twenty microsporangia and representing a staminate flower, is the synandrium.

While the spadix may bear staminate flowers almost to the tip, sometimes its distal end is sterile. The synandria are usually flat-topped but sometimes show centrally located depressions of varying extent, which on evidence from transition forms are regions where pistils would be located.

Both ENGLER and BENTHAM and HOOKER described the staminodes as fused into a ring around the ovary. While this condition has been found, more often one to five separate staminodes to a pistillate flower were noted (fig. 14*ST*). Sometimes the staminodes were so large as completely to fill the spaces between pistillate flowers; at other times they were so inconspicuous as to be covered by the bulging sides of the pistils, and sterile regions of the spadix were then visible between the flowers. Rarely the staminodes of the more distal pistillate flowers bear apparently normal pollen. Staminate flowers may abut directly on the pistillate, but sometimes a transition zone is indicated by relatively few and scattered flowers, or flowers consisting exclusively of staminodes.

The normal pistil (figs. 14, 15) has a slightly flattened globular ovary (*OV*) with a short style (*SE*) and a terminal stigma (*S*), but various types of incompletely developed pistils occur between staminate and pistillate parts of the spadix. In addition to ovules borne normally on the placenta, they were sometimes borne at the base of the style on the protuberance into the ovarian chamber below the top of the ovary. Six substylar ovules were found in an ovary which contained also seven placental ovules.

DEVELOPMENT OF POLLEN

DUGGAR'S observations (**7**) on development of the pollen in *Peltandra* did not cover some of the earlier stages of meiosis, and the writer differs with him on some interpretations. As noted elsewhere (**6**), the chromosome number 22 given by DUGGAR does not appear to refer expressly to either *Peltandra* or *Symplocarpus*.

When preparations made from staminate flowers fixed without previous dissection showed the synizetic phenomenon in the earlier stages of meiosis, it was found necessary to dissect out contents of individual microsporangia in the fixing fluids in order to avoid clumping of the nuclear material. Sporocytes fixed in this way for temporary aceto-carmine mounts, or for imbedding and sectioning by the alcohol-xylol-paraffin method, gave about the same results as fresh untreated sporocytes dissected and mounted in paraffin oil. While it is evident that artifacts may be produced in the material during dissection, and indeed the instances of cytomixis observed are probably the result of tension and pressure, yet the frequent absence of distortion in all the stages, and another aspect of nuclear behavior to be discussed later, encourage confidence in the results obtained.

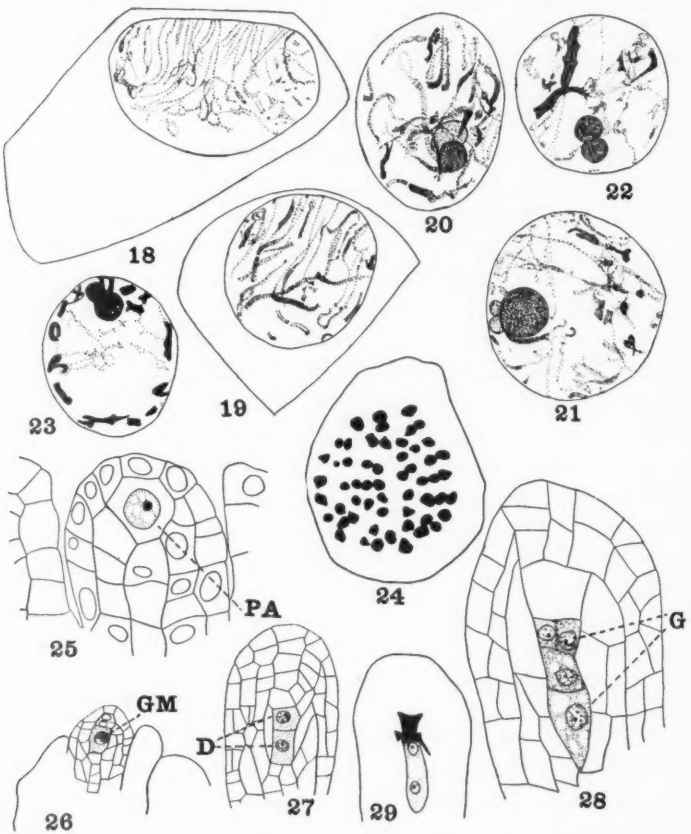
In the microsporangium, as in the ovule, the definitive sporogenous tissue is not marked off until the spring in which the meiotic divisions and flowering occur. Within a month of the occurrence of meiosis, the tapetal, wall, and sporogenous layers can be distinguished. Somatic mitoses occur in the sporogenous cells during the same season that the meiotic divisions occur. The wall is four to seven cells thick, the tapetum two to three, and the sporogenous region four or five in cross section. At length the cells of the sporogenous tissue enlarge, containing larger nuclei and denser cytoplasm than cells of surrounding tissues.

At first the large resting nuclei of the microsporocytes contain four or more nucleoli, stain lightly with Heidenhain's haematoxylin, and appear to contain fine filamentous structures with scattered, more chromatic material (fig. 16). As the nuclei enter the meiotic prophase, bodies similar to prochromosomes are formed; that is, vaguely limited bodies, roughly rectangular in section, and composed—in part at least—of filamentous structures (fig. 17). The delicate chromonemata observed in early meiosis seem to arise from the prochromosome bodies, and later stages show their gradual evolution and the initiation of their orientation. The few denser-staining regions evident in the resting nucleus give way to scattered, darkly staining structures which seem to become incorporated with the fine threads. The nucleoli tend to fuse in pairs. At this point, while dissected material showed a polarization of the threads, other material showed the familiar synizetic ball. Eventually a majority or all of the threads become polarized ("bouquet" stage) and extend parallel to each other from one side of the nucleus. Toward the periphery of the nucleus the chromonemata curve outward. The threads at this stage do not stain deeply and no structure could be discerned inside them, but their arrangement (fig. 18) indicates that they may be pairing. Sometimes there is a definitely thicker strand, which may be an already synapsed thread. More definite conclusions concerning behavior at pairing cannot be formed as yet.

In preparations showing polarization of the chromonemata, orientation of the threads in different nuclei was more or less random, except that frequently in two neighboring nuclei the chromonemata were oriented toward each other, perpendicular to a common cell wall separating them. This tends to emphasize the validity of the method of fixation, since it suggests that the chromonemata may be oriented with respect to the last somatic metaphase plate. The random orientation otherwise observed in any sporogenous mass seems to invalidate the suggestion that the arrangement is the result of an external mechanical disturbance, since if the latter were true, orientation of chromonemata in different nuclei would tend to be uniform and the nuclei otherwise distorted. A similar bouquet stage has been observed in various animals and plants (**4, 5, 14, 19, 24, 31, 32**).

The next stage was frequently observed. The threads, thicker in diameter and

staining more readily and sharply, still show in part the polarized arrangement of the bouquet. Clearly they are fewer in number. Their dual nature is sometimes obvious (fig. 19). The chromonemata do not appear homogeneous but are composed of densely staining granules (chromomeres) separated by less chromatic



FIGS. 18–29.—Figs. 18–24, stages in meiosis in microsporocytes. Fig. 24, polar view of metaphase. Figs. 25–29, longisections of nucellus or distal portion of ovule, showing development of female gametophyte.

regions. Although such bodies were not found in general, their presence was clear in a few cases. The chromonemata at this stage did not show uniform diameter throughout their length. This phenomenon, called first "partial contraction" and more recently "differential condensation," has been noted in animals and plants (4). In *Peltandra*, as in some other angiosperms, it seems to persist from

this late zygotene stage up to the beginning of diakinesis (figs. 19–23). From this time on the nucleoli are usually fused into one body, or closely associated, and contain few to many vacuolar spaces. With this, zygotene is complete and pachytene is gradually entered. It is clear from the number of ends evident in the ensuing stages that the chromonema is not a continuous thread, although the number of elements could not be ascertained. As the chromonemata grow shorter, the so-called spireme stage is reached. Material used for study of this and subsequent stages was not fixed with the same precaution for most rapid penetration as material for preceding stages; hence observations will be reported briefly.

The last traces of polarization vanish and the threads extend throughout the nucleus as the spireme or pachytene stage progresses. Following pachyphase, the chromonemata begin to show marked differences in chromaticity in some regions (figs. 20–22), and as they shorten tend to come into contact. These phenomena are more marked through the diplotene stage. With advanced diplophase (fig. 21) the contracting chromonemata migrate toward the periphery of the nucleus, and their dual nature is plain (fig. 22). Characteristic tetrad shapes appear (fig. 23).

After disappearance of the nuclear membrane the spindle, at first multipolar, becomes bipolar (cf. **7**). The haploid number of chromosomes, as ascertained by the various stages of the heterotypic (fig. 24) and homoeotypic divisions, is 56. The meiotic divisions are "successive." Localization of chromatic material never entirely disappears from the chromosomes at interkinesis, although irregularly outlined nucleoli, one or more per dyad, appear.

As the tetrads of microspores become established, the walls of the tapetal cells disappear and their cytoplasm appears to migrate throughout the locule and to separate the tetrads. Later the nuclei of the tapetal cells leave their peripheral position and become distributed throughout the microsporangium. The individual microspores become rounded off and invested with a heavy, at first unsculptured, wall. The periplasmodial nuclei are amoeboid in form. At maturity, just before the pollen grains are shed, they measure about 25μ in diameter, are sculptured with short, pointed spines, and contain a large nucleolated pollen tube nucleus and two smaller (male) nuclei devoid of nucleoli and staining densely. The wall of the mature pollen grain is pitted. The pollen-tube nucleus, which becomes lightly staining, irregular in outline, and apparently degenerate, may precede or follow the male nuclei into the pollen tube. Tests showed abundance of starch grains in mature pollen grains and pollen tubes. The male nuclei usually have surrounding hyaline areas clearly delimited from the granular cytoplasm of the tubes (cf. **29**). The possibility that these hyaline areas are artifacts has not been overlooked. Sometimes a distinct granular cytoplasmic layer can be seen surrounding each male nucleus, and this in turn is surrounded by the clear areas.

At maturity the sessile, staminate flowers consist of an angular disk 3–5 mm.

long, 2–3 mm. wide, and about 1 mm. high. In a tangential section of the spadix a sharp cusp appears between the upper portions of the sporangia of each of the pairs arranged around the periphery of the floral disk. This cusp marks the sole region in which the cells of the hypodermal layer of the theca remain small, with walls unthickened. The one-cell layer envelope of mechanically strengthened cells also occurs on the side of the loculus attached to the floral disk. As the periplasmodium of each microsporangium and the septum separating the microsporangia of each theca disappear in the course of development, the pollen masses become confluent. The wall of the theca ruptures in the region of the unstrengthened hypodermal cells. At first the freshly shed pollen tends to remain in vermiform masses near its place of exit on the outer surface of the synandrium, but on being disturbed most of it falls into the dilated proximal part of the spathe.

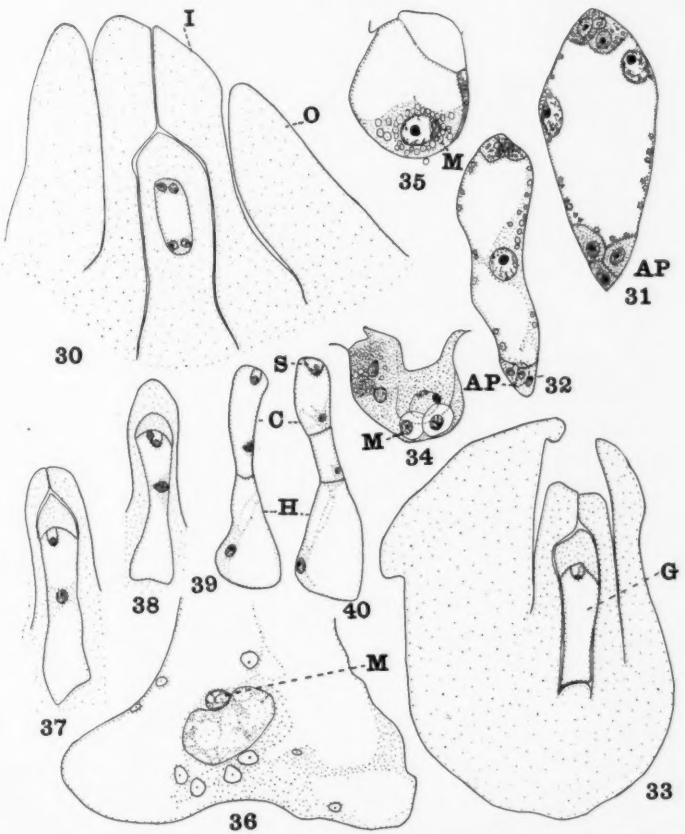
Development of megagametophyte

The primordia from which the one to ten ovules arise grow upward and outward from the edge of the basal placenta and eventually approach the wall of the ovary in its upper regions. At the first sign of the integuments, the primary archesporial cell is not clearly marked off. The inner integument differentiates more rapidly than the outer. A median hypodermal cell of the nucellus is differentiated as the primary archesporial cell (fig. 25*PA*). The megaspore mother cell (fig. 26*GM*) can be seen with two overlying tapetal cells, which were probably formed by division of the sister cell of the megaspore mother cell. *Symplocarpus* (**27**) and *Calla* (**23**) also show tapetal cells, but other aroids—for example, *Acorus calamus* (**1**)—form none. Throughout development of the ovule, the portion of the outer integument on the side next to the funiculus is larger than that diametrically opposite. The micropylar end of the hemianatropous ovules is consistently directed outward toward the wall of the ovary (fig. 15*M*).

By the time the first meiotic division has occurred the megaspore mother cell is well surrounded by nucellar tissue, and the distal part of the inner integument is growing contripetally and overarching the nucellus, forming the narrow micropyle. At first the outer integument does not keep pace in growth longitudinally with the inner, but it is broader than the inner integument, which is two or three cells thick, except at its tip. As in other aroids (**23**), the inner layer of the inner integument forms a distinct layer of cells, rectangular in outline, with their longer axes perpendicular to the longitudinal axis of the ovule (the "tapetal" layer of the megagametophyte).

The spindle in the smaller, micropylar member of the dyads (fig. 27*D*) is perpendicular to, and the spindle in the chalazal dyad (fig. 28) is parallel to, the longitudinal axis of the ovule. Of the four megaspores formed (fig. 28*G*), the chalazal one becomes conspicuously vacuolated and gives rise to the megagameto-

phyte. The outer integument has now reached the length of the inner and begins to exceed it. Centripetal growth of the distal end of the inner integument has reduced the micropylar opening. The chalazal megaspore seems to develop nor-



FIGS. 30–40.—Figs. 30–33, longisections showing development of female gametophyte. Fig. 30, section of micropylar portion of ovule perpendicular to longitudinal axis of funiculus (*O*, outer; *I*, inner integument). Fig. 33, longisection of ovule at fertilization, parallel to longitudinal axis of funiculus. Fig. 34, male nuclei beside egg cell. Fig. 35, fertilization. Fig. 36, male nucleus beside fusion nucleus in chalazal region of female gametophyte. Fig. 37, gametophyte after fertilization. Figs. 38–40, first and second divisions in endosperm.

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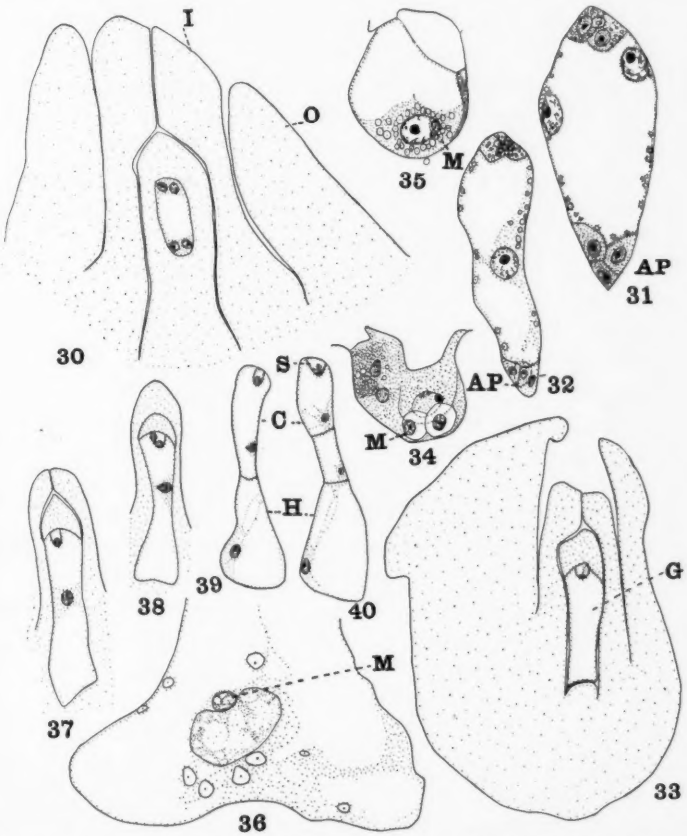

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phyte, after formation of antipodal cells and the egg apparatus. Three antipodal cells are organized (figs. 31, 32, *AP*). They soon show signs of degeneration.

In the later stages of its development the megagametophyte contains abundant starch grains, especially in the micropylar cells and about the fusion nucleus. The nucellar tissue flanking the megagametophyte has begun to disappear (through digestion) by this time. Before fertilization occurs this part of the nucellus has been completely digested, and the sides of the megagametophyte (fig. 33*G*) are in contact with the inner layer of the inner integument.

After degeneration of the antipodals the fusion nucleus comes to lie at the chalazal end of the megagametophyte, which becomes truncate as it extends coincident with digestion of the chalazal nucellus. When ready for fertilization, the megagametophyte contains two synergids and an egg cell in the micropylar end and the fusion nucleus in the opposite end. The outer integument now extends beyond the inner. After the chalazal megaspore has begun to enlarge, or sometimes even before, a palisade of unicellular glandular hairs develops from the superficial layer of the placenta and adjacent regions of the funiculi. These hairs, together with those formed within the ovary near the base of the style, produce a jelly-like, colorless material that fills the ovary before fertilization and persists until after the seed germinates.

Pollination and fertilization

Prior to pollination the spathe remains tightly wrapped about the spadix, one edge overlapping the other. The upper part of the spathe surrounding the staminate flowers becomes marked off from the lower surrounding the pistillate flowers. While the upper two-thirds of the spathe is dark green and tapers gradually toward the tip, the lower part is yellowish to light green and of greatest diameter at the middle. Opening in both regions is preceded by longitudinal extension of first the inner and then the outer margin of the spathe, both margins being thrown into wavelike folds. The lower part of the spathe opens first, but the opening is small and only a few pistillate flowers can be observed from without. Each pistillate flower consists of one to five whitish, fleshy staminodes surrounding a flask-shaped pistil about 3 mm. high with a subglobose, one-celled ovary about 2.5 mm. in diameter; a comparatively thick style 0.8–1.5 mm. long, having a central canal lined with hairs; and an inconspicuous terminal stigma of short unicellular hairs (figs. 14, 15). The upper portion of the spathe opens later. Sometimes it opens completely, freely exposing the tip of the spadix; but it may only loosen a little, without actually exposing the staminate flowers.

The behavior of the spathe and other circumstances make cross-pollination feasible and probable. Insects (Syrphidae, Chloropidae, and others) were seen entering the spathe, frequently laden with *Peltandra* pollen. Eggs of insects are

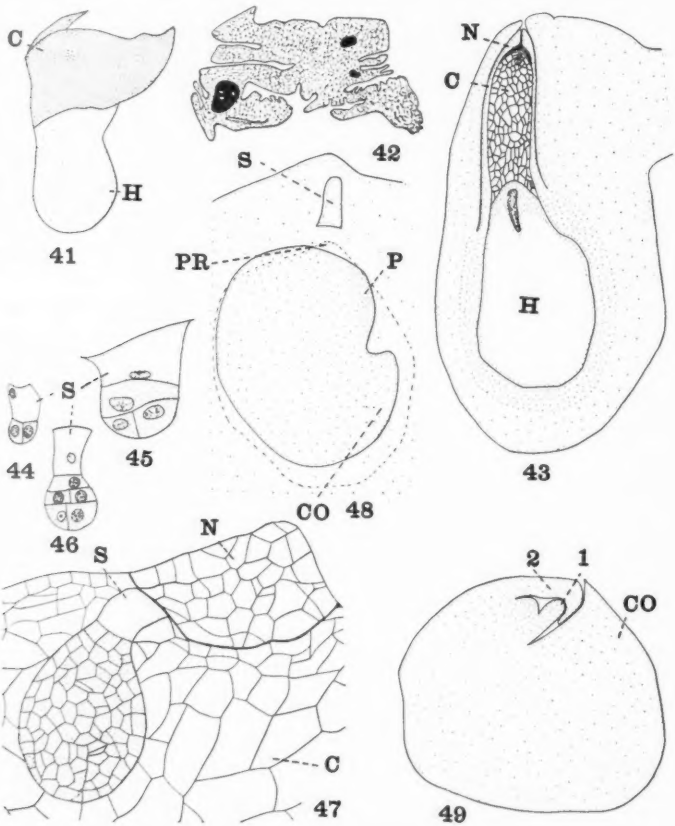
often found on the inner surface of the spathe, and insect larvae develop in and feed on the immature fruiting spadix. In several instances, when only the lower part of the spathe surrounding the pistillate flowers was open, germinated pollen grains were observed on the stigmas of the pistils before there was any sign of dehiscence and liberation of pollen from the loculi of the synandria on the same spadix. Apparently the pistillate flowers can be and are pollinated before the staminate ones on the same spadix liberate pollen. Should cross-pollination of any pistillate flower fail to occur, self-pollination is still possible, if the stigma remains receptive; for large amounts of pollen from the overlying synandria are liberated and reach the lower region of the spadix (by means of insects or force of gravity) after fertilization has occurred in some of the pistillate flowers on the same spadix.

Germinated pollen grains have been observed on the stigma; pollen tubes have been found in the stylar canal, in the jelly-like material within the ovary, entering the micropyle, and traversing the micropylar nucellus. As recorded for several other plants (**29**), more than one pollen tube may enter the megagametophyte. In one case three pollen tubes entered a single micropyle. In some instances clear areas, like those observed in the pollen tubes, surround the male nuclei in the megagametophyte (fig. 34*M*). One male nucleus (fig. 35*M*), lacking a nucleolus and staining densely, fuses with the egg nucleus; a second fuses with the fusion nucleus (fig. 36*M*). Among aroids double fertilization has also been recorded for *Arum maculatum* (**21**) and *Arisarum vulgare* (**22**), and suggested for *Dieffenbachia picta* var. *baraquiniana* (**16**). The fusion nucleus contains a large vacuolated nucleolus. Upon fusion with a male nucleus, the resulting endosperm nucleus finally comes to possess several smaller nucleoli, and the rather irregular chromatic reticulum is replaced by a more threadlike structure.

Development of endosperm

So far as observed, the endosperm nucleus always divides before the oospore nucleus. Usually this nucleus migrates from its chalazal position toward the micropyle (fig. 37), dividing at a position somewhat more than half way (fig. 38). Cytokinesis follows this karyokinesis (fig. 39). In this respect *Peltandra* resembles some aroids (**1, 20, 23**) but differs from others (**15, 23, 26, 27**). The micropylar endosperm cell thus formed gives rise to a cellular endosperm (figs. 39, 40, 43, *C*). The chalazal cell (*H*) never divides again and eventually reaches a tremendous size (figs. 41, 43, *H*), 2.4 by 1.6 mm. or more (cf. **26**). Its nucleus also fails to divide again, increases greatly in size, and becomes very much lobed (fig. 42). After attaining a large size, the nucleolus fragments and the pieces become highly vacuolated. When endosperm with the chalazal cell still intact was dissected from ovules, mounted in tap water, and observed with the microscope, large coarse strands of cytoplasm were noted traversing the cell vacuole of the giant haustorial

cell. Within these strands the middle regions could be seen in streaming motion. Large haustorial cells, similar in origin and possessing large and intricately lobed nuclei, have been reported for other aroids, but the number found in different species varies from one to eight.



FIGS. 41–49.—Fig. 41, endosperm dissected from ovule, with haustorial cell intact. Fig. 42, section of nucleus of haustorial cell. Fig. 43, longisection of maturing ovule in plane of funiculus. Figs. 44–49, development of embryo (*N*, nucellar cap).

The second nuclear division in the endosperm, that is, the division of the nucleus of the micropylar daughter cell, is also accompanied by cytokinesis (figs. 39, 40); hence development of the endosperm is cellular. The occurrence of cellular endosperm has been suggested or reported several times for aroids (**23, 29, 30**). Usually the reports are uncertain or the necessary sequence of stages has not been

obtained. On the other hand, the reports indicate that if the endosperm (especially the micropylar portion) is not cellular from the start, the non-cellular condition is of brief duration (**1**, **25**).

The third division in the endosperm was not observed. Soon walls, perpendicular to those first formed and parallel to the long axis of the ovule, appear in the chalazal region of the cellular endosperm. Eventually the micropylar endosperm cells also undergo divisions in this plane.

In the development of the ovule after fertilization, the haustorial cell at first enlarges more rapidly than the cellular endosperm, especially in a direction perpendicular to the long axis of the ovule. Consequently the products of the megagametophyte (and the entire ovule) assume, as a whole, a pyriform shape, the micropylar end being narrower (fig. 43). In subsequent development of the ovule, the haustorial cell, after attaining relatively great dimensions, ceases to enlarge; it is overtaken and finally surpassed in cross-sectional area, as well as in longitudinal extent, by the cellular endosperm. By the time the embryo sporophyte becomes fairly conspicuous the form of the maturing seed is again pyriform, but this time the chalazal region housing the haustorial cell is the narrower. In the ripe seed the micropylar portion containing the large embryo is by far the more conspicuous; the haustorial cell with its surrounding coat of parent sporophytic tissue becomes a mere withered protuberance of the chalazal region.

Development of embryo

When the endosperm is in the 2- or 3-cell stage, the oospore nucleus divides and a wall perpendicular to the longitudinal axis of the ovule is formed (fig. 40). The micropylar cell is the larger, and with the possible exception of one preparation was not observed to undergo further nuclear or cellular divisions (figs. 40, 44, 45, 46, 47, 48, *S*). It becomes the one-celled suspensor, and although relatively large attains no striking dimensions or differentiation. The smaller distal cell gives rise to the embryo proper, and next divides by a wall perpendicular to the first (fig. 44).

A review of studies on aroids reveals that while the suspensor does not become conspicuous, its origin and the fate of the micropylar cell of the 2-celled proembryo vary in different species. *Pistia* (**20**) has no suspensor; in *Arum orientale* and *A. maculatum* (**20**) the suspensor arises from the micropylar cell of the 2-celled proembryo; in *Acorus* (**1**) and *Antherurus* (**17**) the micropylar cell divides by a transverse wall; in *Calla* (**23**) and *Arisaema* (**26**) cellular suspensors arise from the micropylar cell.

The first and second cell walls to be formed in the embryo are in some cases parallel to each other and perpendicular to the long axis of the ovule, for example, in *Aglaonema* (**2**) and others. In *Peltandra*, *Arisaema* (**26**), and *Spathicarpa sagit-*

taefolia (3) the second wall in the proembryo is perpendicular to the first and parallel to the long axis of the ovule. The immediately succeeding divisions were not seen, but the 7-cell stage (fig. 45) indicates that the next walls formed are parallel to the first and hence perpendicular to the second. Figure 46 is a longitudinal section of the 10-cell stage.

The embryo proper now proceeds to form a globular to ovoid body (fig. 47). Later a groove appears on one side of the embryo and separates the terminal cotyledon (fig. 48*CO*) from the lateral plumule primordium (fig. 48*P*; dotted line is outline of embryo in region of primary root, *PR*). Except in the region separating the plumule from the cotyledon, the surface of the latter is evenly continuous with the rest of the embryo at this as well as at later stages of embryonic development, for the radicle does not form a distinct protuberance. Initiation of the primary root begins shortly after the cotyledon and plumule primordia have been differentiated.

While the radicle seems to undergo an abortive development, the terminal cotyledon enlarges so rapidly and extensively that the plumule comes to lie roughly at the micropylar end of the embryo as a whole. The growing point of the plumule always points laterally, is usually distinct; and as the cotyledon enlarges, gives rise to the first cauline foliar organ on its micropylar side (fig. 49, *2*). The next leaf arises on the chalazal side (fig. 49, *1*). At maturity the embryo possesses a relatively large plumule with six or seven cauline leaf rudiments and several rudimentary adventive roots.

In the late summer and fall the lower parts of the spathes around the ripening fruits disintegrate, together with the remaining axial part of the spadix. Since the released fruits are buoyant and the marshes occasionally flooded, they were observed dispersed by flowing water in Maryland.

Summary

1. The subterranean vertical rhizome of *Peltandra virginica* is built up sympodially from branches arising in the axils of the penultimate leaves of continuation shoots of successively higher rank. Each continuation shoot, as well as the primary axis, terminates in a spadix. A second spadix arises in the axil of the ultimate leaf.

2. Usually vegetative buds, which sometimes result in vegetative multiplication of the plant, arise in the axils of only the antepenultimate leaves.

3. There is a tendency to limit the number of foliage leaves formed on a continuation shoot. The minimum number was three.

4. Leaves and inflorescences appear to be initiated about 20 months before they actually emerge from the terminal bud.

5. Development of the staminate flowers differs from that of the pistillate in that the latter alone show a distinct segmentation of the floral primordia.

6. A "bouquet" stage was found in early meiosis. The haploid number of chromosomes was 56.

7. Periplasmodium formation was found in the microsporangium, and the pollen is 3-nucleate before it is shed. Evidence for the occurrence of cross-pollination was found. "Double" fertilization occurs.

8. The endosperm is cellular from the start; the large haustorial cell in the maturing ovule is the chalazal daughter cell formed upon division of the endosperm cell.

9. The embryo contains a large, well-developed plumule and a small, poorly-developed primary root.

The writer wishes to acknowledge his debt to the late Professor D. S. JOHNSON, under whom this work was undertaken, and to Professor C. O. ROSENDAHL, for helpful criticism of the paper; and also his appreciation for the cooperation of Miss ALMA RUTLEDGE, Mr. MILTON SEIDMAN, and Dr. T. I. EDWARDS. Insects were identified by Dr. ELIZABETH FISHER.

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LITERATURE CITED

1. BUELL, M. F., Embryogeny of *Acorus calamus*. BOT. GAZ. **99**:556–568. 1938.
2. CAMPBELL, D. H., Studies on the Araceae. Ann. Botany **14**:1–25. 1900.
3. ———, Studies on the Araceae. Ann. Botany **17**:665–687. 1903.
4. DARLINGTON, C. D., Recent advances in cytology. 2d ed. Philadelphia. 1937.
5. DÖPP, W., Die Apogamie bei *Aspidium remotum* Al. Br. Planta **17**:86–152. 1932.
6. DUDLEY, M. G., Morphological and cytological studies of *Calla palustris*. BOT. GAZ. **98**: 556–571. 1937.
7. DUGGAR, B. M., Studies in the development of the pollen grain in *Symplocarpus foetidus* and *Peltandra undulata*. BOT. GAZ. **29**:81–97. 1900.
8. EDWARDS, T. I., The germination and growth of *Peltandra virginica* in the absence of oxygen. Bull. Torr. Bot. Club **60**:575–581. 1933.
9. ———, Seed frequencies in *Cytisus* and *Peltandra*. Amer. Nat. **68**:283–286. 1934.
10. ENGLER, A., Vergleichende Untersuchungen über die morphologischen Verhältnisse der Araceae. Verhandl. ksl. Leop.-Carol. Deut. Akad. Naturforscher **39**:159–232. 1877.
11. ———, Beiträge zur Kenntniss der Araceae. V. Bot. Jahrb. **5**:141–188; 287–336. 1884.
12. ———, Das Pflanzenreich. IV. 23A. Araceae, Pars Generalis. Leipzig. 1920.
13. ERTL, P. O., Vergleichende Untersuchungen über der Entwicklung der Blattnervatur der Araceen. Flora **126**:115–248. 1932.
14. GEITLER, L., Grundriss der cytologie. Berlin. 1934.

15. GOW, J. E., Embryogeny of *Arisaema triphyllum*. BOT. GAZ. **45**:38–44. 1908.
16. ———, Studies in Araceae. BOT. GAZ. **46**:35–42. 1908.
17. HANSTEIN, J., Die Entwicklung des keimes der Monokotylen und Dikotylen. Hanstein's Bot. Abhl. **1**:1–112. 1870.
18. HART, H. T., Delayed germination in *Peltandra virginica* and *Celastrus scandens*. Publ. Puget Sound Biol. Sta. **6**:254–261. 1928.
19. HEDAYETULLAH, S., Meiosis in *Oenothera missouriensis*. Proc. Roy. Soc. London B. **113**: 57–70. 1933.
20. HOFMEISTER, W., Neue Beiträge zur Kenntniss der Embryobildung der Phanerogamen. II. Monokotyledonen. Abh. Kön. sächs. Ges. Wiss. **7**:629–760. 1861.
21. JACOBSON-PALEY, R., Sur le haustorium et la formation de l'albumen dans l'*Arum maculatum*. Bull. Soc. Bot. Geneve, Ser. 2. **12**:55–64. 1920.
22. ———, Sur le succoir de l'*Arisarum vulgare* Targ.-Tozz. et le rôle de la région chalazienne du sac embryonnaire. Bull. Soc. Bot. Geneve, Ser 2. **12**:65–86. 1920.
23. JÜSSEN, F. J., Haploid generation der Araceen. Bot. Jahr. **62**:155–283. 1928.
24. KAUFMANN, B. P., The existence of double spiral chromatin bands and a bouquet stage in *Tradescantia pilosa* Lehm. Amer. Nat. **59**:190. 1925.
25. MICHELL, M. R., The embryo sac of *Richardia africana* Kth. BOT. GAZ. **61**:325–326. 1916.
26. PICKETT, F. L., A contribution to our knowledge of *Arisaema triphyllum*. Mem. Torr. Bot. Club **16**:1–55. 1915.
27. ROSENDAHL, C. O., Embryo sac development and embryology of *Symplocarpus foetidus*. Minn. Bot. Studies **4**:1–9. 1909.
28. ———, Observations on the morphology of the underground stems of *Symplocarpus* and *Lysichiton*, etc. Minn. Bot. Studies **4**:137–152. 1911.
29. SCHNARF, K., Embryologie der Angiospermen. Linsbauer's Handbuch der Pflanzenanatomie 10, II. Berlin. 1929.
30. ———, Vergleichende Embryologie der Angiospermen. Berlin. 1931.
31. SZAKIEN, B., Prophase meiotique dans l'*Equisetum silvaticum* et l'*E. palustre*. La Cellule **45**:16–26. 1936.
32. WILSON, E. B., The cell. 3d ed. New York. 1925.

COAL FLORA STUDIES: LEPIDODENDRALES[1]

FREDDA D. REED

(WITH THIRTY-FIVE FIGURES)

Introduction

In the century or more since the appearance of LINDLEY and HUTTON'S (10) account of histological studies of *Lepidodendron*, considerable literature has accumulated on the anatomy and morphology of Paleozoic arborescent lycopods. Nevertheless, there is still lacking precise knowledge of specific details of both vegetative and reproductive structures that would serve, not only to give a more complete picture of the group, but also to provide diagnostic criteria for identifying to generic and specific position isolated portions of these fossil plants. The present state of our knowledge does not discriminate between *Lepidodendron* and *Sigillaria* in the case of many of the detached and fragmented lycopodiaceous organs, and for that reason the term Lepidodendrales, an order including all the arborescent lycopods, is used in the above title.

The material here described appeared in coal balls from Harrisburg, Illinois, coal seam no. 5, which is in the Alleghany group of the Upper Pennsylvanian. The coal balls were collected under the auspices of the Illinois State Geological Survey by the late Dr. A. C. NOÉ, who kindly turned them over to me for investigation. The preparations made from them, consisting of thin sections, opaque sections, and celloidin peels, will be deposited with the Noé collection of paleobotanical specimens in the Illinois State Museum at Urbana, Illinois. In these Harrisburg coal balls there are, in addition to the forms to be described here, many plant fragments some of which have already been recorded (**8, 12, 13, 14**), and there are still other genera awaiting description.

Of the variety of organs, leaves, stems, roots, and sporangia selected for recording and description here, none of them were found in organic association; moreover, none of them were preserved in entirety. They were chosen because all are genera of the Lepidodendrales order and because in every instance certain of the tissues are well preserved.

Observations

I. LEAVES

In the following descriptions only the anatomy of the leaf blade is considered. In many instances a leaf could be followed through several serial sections, a dis-

[1] Presented before the Paleobotanical Section of the Botanical Society of America at Columbus, December, 1939.

tance of 1–3 cm. In a few there was some diminution of size toward the apex, but in none of the leaves were both extremities found; hence there was no indication of the length, and—save for the decrease in diameter at the apical region—there were no observations on histological modifications from the base to the apex.

The term *Lepidophyllum* is here applied to those Lepidodendralean leaves which have a single xylem strand. It was formerly thought that such leaves belonged to *Lepidodendron*, whereas those with a double strand belonged to *Sigillaria*. More recently GRAHAM (5) has observed: "There does not appear to be any fundamental difference between the leaves of *Sigillaria* and *Lepidodendron*. All leaves with a double bundle are attributed to *Sigillaria*, but this character is not constant for that genus."

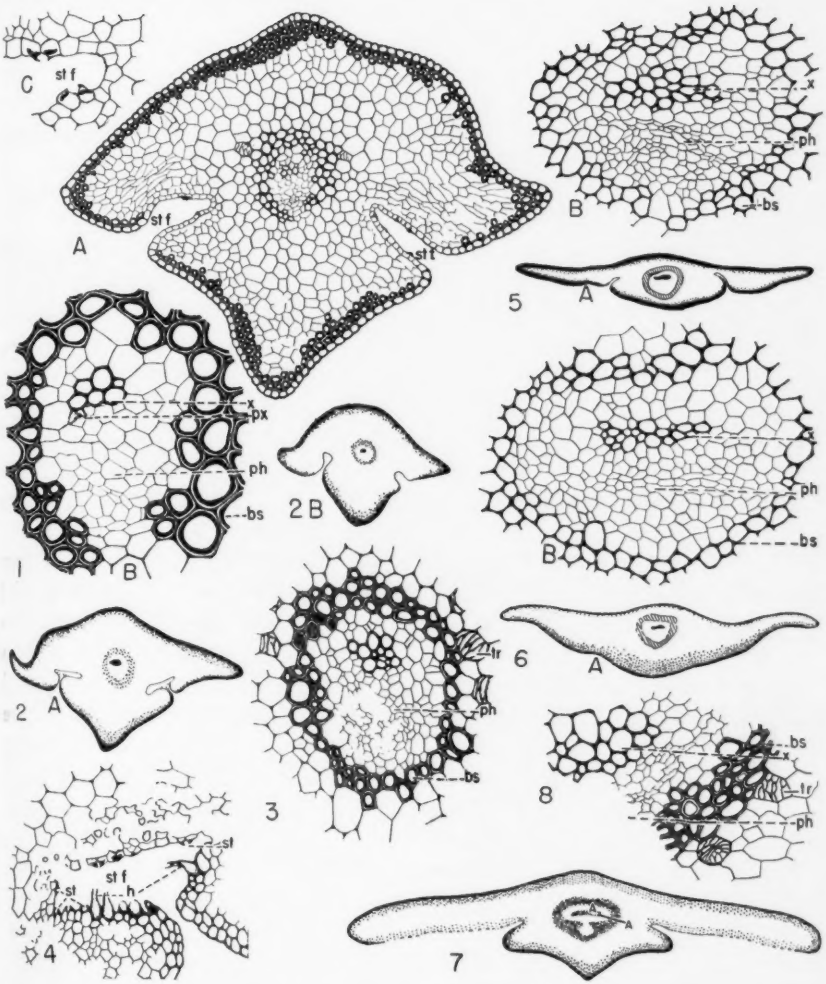
Lepidophyllum aciculum sp. nov.

DIAGNOSIS.—Leaves acicular, 0.75 mm. broad by 0.59 mm. thick. Ventral surface convex; dorsal surface convex to angular with two lateral furrows and conspicuous median keel. Vascular strand exarch to mesarch, collateral, surrounded by bundle sheath. Some of cells lateral and exterior to bundle sheath differentiated into transfusion tissue. Mesophyll with no differentiation into palisade cells, mostly compact, but lacunar in region of stomatiferous furrows. Hypodermis one to three cells deep except in region of furrows, where absent. Epidermis of relatively thick-walled cells; stomata confined to furrows.

If there is a lepidodendroid leaf in the Harrisburg material that might be regarded as typical it is this one, for almost every coal ball containing leaves has some fragments of it. The leaf is fine and needle-like; in its known dimensions it is comparable with the leaves of *Pinus strobus*.

The single xylem strand consists of six to eight elements with scalariform markings. The protoxylem was not definitely determined, but on the abaxial side there is some irregularity of cells, either from incomplete preservation or from some disintegration while the plant was living, and that irregularity may represent the position of the protoxylem (fig. 1 B*px*). The vascular bundles of *Lepidophyllum* have been interpreted as concentric (5), but in this leaf, as in the succeeding ones, below the xylem there is a group of thin-walled cells, irregular in size, with the aspect of phloem tissue (fig. 1 A, B*ph*). Surrounding the xylem and phloem is a layer, one or two cells deep, of large thin-walled parenchyma, and this layer in turn is succeeded by the thick-walled fibrous cells of the bundle sheath, three to four cells deep. In the leaf illustrated the bundle sheath is not complete on the abaxial side (fig. 1 B). For the most part the mesophyll is of undifferentiated thin-walled cells with few and small intercellular spaces, yet in the marginal region the cells are slightly elongated and the intercellular spaces are more conspicuous.

The hypodermis, of small, regular, thick-walled, and isodiametric cells, sur-



FIGS. 1–8.—*Lepidophyllum:* Fig. 1, *L. aciculum*. A, transection of leaf: *st f*, stomatal furrow. B, vascular strand of A: *x*, xylem; *px*, protoxylem; *ph*, phloem; *bs*, bundle sheath. C, detail of stomatal furrow at left of A. Noé coll. H 236 1. Figs. 2–4, *L. trichosulcata*. Fig. 2, diagrams of transections: A, median section; B, same leaf from apical region. Noé coll. H 227 A 22. Fig. 3, detail of vascular strand of 2 A: *tr*, transfusion tissue. Fig. 4, detail of stomatal furrow at right of 2 A: *st*, stoma; *h*, epidermal hair. Fig. 5, *L. alatum:* A, diagram of transection; B, detail of vascular strand. Noé coll. H 236 13. Fig. 6, *L. latifolium:* A, diagram of transection; B, detail of vascular strand. Figs. 7, 8, *L. thomasi*. Noé coll. H 236 12. Fig. 7, diagram of transection. Fig. 8, detail from A——A of fig. 7.

rounds the mesophyll save for the region of the furrows, but it is thickest above and below the vascular strand and at the margins (fig. 1 A). The epidermis is likewise of small regular cells, but the walls are not so thick as are those of the hypodermis. About the furrows the walls are thinner, and here are found the stomata (fig. 1 C). These were apparently crowded, for often there is only one epidermal cell separating two guard cells.

Lepidophyllum trichosulcata sp. nov.

DIAGNOSIS.—Leaves about twice as wide as thick, 1.8 mm. by 0.94 mm., tapering to 1 mm. by 0.7 mm. Ventral surface convex; dorsal surface angular with conspicuous median keel and two lateral stomatiferous furrows. Vascular strand exarch to mesarch, collateral, and inclosed by bundle sheath of thick-walled fibrous cells. Transfusion elements in tissue surrounding bundle sheath. Mesophyll thin-walled, irregular cells but not differentiated into palisade tissue; larger lacunar areas in region of stomatiferous furrows. Hypodermis two to four cells deep except around furrows, where absent. Epidermis of relatively small and regular cells, except in furrows, where their walls are thinner and some cells are differentiated into guard cells, others into hairs which extend into the furrows.

While the general aspect of *Lepidophyllum trichosulcata* differs markedly from that of *L. aciculum*, yet a comparison of the diagnoses reveals but two essential differences: (1) the difference in size and shape, which would of itself not be considered a specific character but in combination with structural differences does have some diagnostic value; and (2) the cellular organization about the lateral furrows. *L. trichosulcata* in its dimensions of width and thickness measures more than twice that of *L. aciculum*. The leaf was observed in several sections, in the last of which is the apex; following it through it is seen to taper gradually to dimensions of about half that of the maximum size and to vary somewhat in the shape of the transverse section (fig. 2 A, B).

The xylem in section (fig. 3) is a circular to elliptical group of twelve to twenty tracheids, the number diminishing toward the apex. In this bundle, as in so many of the specimens of *Lepidophyllum*, the tissue below the xylem is incompletely preserved. The remaining cells are somewhat irregular in shape and are thin walled. They were not observed in longitudinal section, and sieve tubes have not been demonstrated; but—as in the former leaf—it is this tissue that is interpreted as that of phloem. Surrounding the xylem and phloem are two to three layers of parenchymatous cells and then the sheath of thick-walled fibrous cells (fig. 3). Exterior to the bundle sheath are occasional transfusion elements with reticulate to scalariform thickenings (fig. 3*tr*).

Neither the cells of the mesophyll nor those of the hypodermis show much differentiation, nor are the two regions sharply delimited from each other. The cells of the hypodermis are more regular but their walls are only slightly thicker.

The most distinguishing feature of this leaf is the cellular organization about the stomatiferous furrows. Many of the epidermal cells in the vicinity of the opening are elongated into hairs which extend into the chamber (fig. 4). Distributed all around the remaining part of the furrow are the stomata, numerous and crowded—eight are indicated at the level shown in figure 4. The hairs, fine and delicate, measuring about 6 μ in diameter, are demonstrable only under oil immersion lens. In transverse section the stomatal furrows or grooves bear some resemblance to the stomatal chambers of *Nerium*, except that in *L. trichosulcata* the hairs are assembled near the opening of the groove as if to guard the entrance and are not interspersed irregularly among the stomata as are those of *Nerium*. The whole mechanism would appear to be very effective in guarding against excessive water loss.

Lepidophyllum alatum sp. nov.

DIAGNOSIS.—Leaf more than five times as wide as thick, 2.25 mm. wide by 0.42 mm. thick. Ventral surfaces slightly convex; dorsal surface convex with two lateral furrows. Vascular strand probably exarch, collateral, inclosed by bundle sheath. Xylem strand in transverse section of about twenty elements arranged in horizontally elongated group. Mesophyll undifferentiated, surrounded by hypodermis, except in region of lateral furrows. Stomata not observed, but thin-walled epidermis and absence of hypodermis about the lateral furrows would indicate that they were confined to that region.

This leaf (fig. 5 A, B) is broader and thinner than that of the preceding two species. In its dimensions of breadth and thickness it is comparable with *Taxus canadensis;* also, these dimensions are in about the same proportions to *L. aciculum* and *L. trichosulcata* as are the dimensions of *T. canadensis* to *Pinus strobus* and *P. rigida*, respectively.

Except for the difference in size and shape and the horizontal arrangement of the xylem elements, this leaf is not especially noteworthy.

Lepidophyllum latifolium Graham

The type specimen of *L. latifolium*, described by GRAHAM (**5**), came from the Lower Coal Measures of Great Britain and is in the Binney collection, Sedgewick Museum, Cambridge.

The size of the Harrisburg leaf, 2.4 mm. wide by 0.5 mm. thick, is somewhat smaller than that of the type specimen, but it lies within the limits of variability as recorded for the species. In both leaves, this (fig. 6 A, B) and the type specimen, the xylem in section is in a horizontally elongated band. While there are more elements in this leaf (twenty xylem elements shown in fig. 6 B), yet the variation in number is not greater than might be found in one strand in its course from base to apex. The disposition of the hypodermis of the two leaves is the same,

in that it is absent, or one cell thick, on the adaxial side of the leaf and from five to six cells thick on the lower side. In other details there is so little variation from the type specimen that there seems no valid reason for setting the Harrisburg leaf apart into a new species.

Lepidophyllum thomasi Graham

The species *L. thomasi* was established by GRAHAM (**5**) for the reception of some lycopodiaceous leaves which were also from the Lower Coal Measures of Great Britain. Of all the specimens of *L. thomasi* in the Harrisburg collection, there is none entirely comformable with the type specimen; however, the kind as well as the degree of variation appears to be individual rather than specific.

The leaf of *L. thomasi* is the largest of the lepidodendroid leaves discovered in this particular collection of coal balls. The length is certainly several centimeters, and some of the transverse sections measure up to 6 mm. in width and 2.5 mm. deep. Usually they are about four times as wide as deep.

There are two distinguishing features that make *L. thomasi* recognizable, even though the leaves may be folded or crushed, partially preserved or fragmented; they are the very characteristic hypodermis and the pattern of the vascular strand. The hypodermis is a sharply defined and massive layer six to ten cells deep. It is thickest above and below the midrib and less well developed on the lower side of the lamina (fig. 7). The cells in transverse section are isodiametric and regular, thick walled, and arranged in radial rows; in tangential section all, except those of the lower side of the lamina, are elongated.

In keeping with the superficial dimensions of this leaf, the vascular strand is larger and composed of more elements than are the strands of the preceding species. The xylem elements, numbering up to fifty or more in the larger bundles, are arranged in a horizontally elongated group as in *L. alatum* and *L. latifolium*. The position of the protoxylem is uncertain, but there are smaller elements at either end of the metaxylem which may be those of the protoxylem (figs. 7, 8). The xylem is surrounded by a tissue of thin-walled irregular cells which is considerably more extensive on the abaxial side (figs. 7, 8); it is this tissue that is labeled phloem.

The bundle sheath and inclosing elements distinguish the vascular strand. Laterally and adaxially the sheath is an elliptical mass of thick-walled fibrous cells (fig. 7), appearing much as in other species of *Lepidophyllum*. On the abaxial side, however, it extends downward into a U-shaped pattern. The U is filled with thin-walled parenchymatous tissue, tissue that may be secretory in nature, as has been described in other instances (**4, 1, 20**), or may be—as sometimes appears—simply a continuation or extension of the phloem. In addition to the two species of leaves described by GRAHAM (**5**), *L. thomasi* and *L. papillonaceum*, this same type of

bundle sheath, although not always interpreted as such, has been found in the leaf traces of *Lepidodendron harcourtii* (1), in the leaf traces of *Lepidophlois fuliginosus* (20), and in the cone scale of *Lepidostrobus* (4). Also CHRYSLER has figured a leaf labeled *Sigillaria*, from the Foot Mine, Shore, Littleborough, Lancashire, which shows the same organization of thick-walled elements about the conducting strand (4).

Sigillariopsis Renault

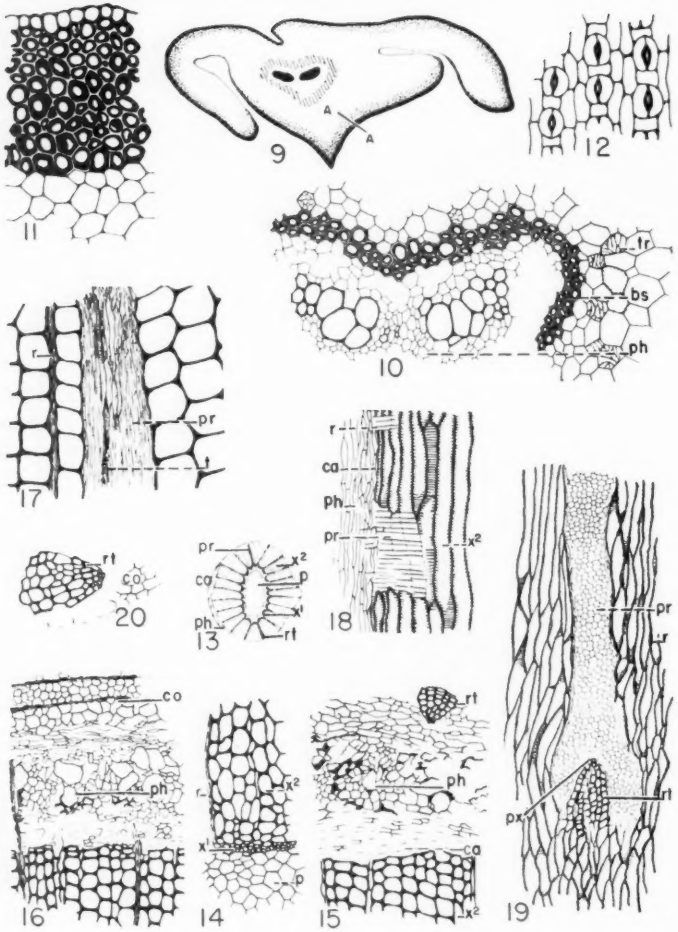
The genus *Sigillariopsis* was established in 1879 by RENAULT (15) for a small stem with leaves attached from the Permian of Autun. Both stem and leaf were essentially lycopodiaceous, the leaf differing from previously described genera in the possession of two parallel xylem strands. In 1904 SCOTT (18) applied the term *Sigillariopsis* to some lycopodiaceous leaves with two xylem strands from the Lower Coal Measures of Lancashire; these leaves were described under the specific name of *S. sulcata*. Subsequently the genus *Sigillariopsis* has become a repository for leaves with a double xylem strand (2, 7, 5), but in only one instance has a specimen been found in close association with *Sigillaria* (2).

Sigillariopsis cordata sp. nov.

DIAGNOSIS.—Leaves broad with thin laminal margins, 3 mm. wide by 1 mm. thick at midrib and 0.25 mm. thick in marginal region. Ventral surface slightly convex, with shallow median depression; dorsal surface with prominent median keel and lateral stomatiferous furrows. Central vascular region has two parallel strands of xylem with smaller elements at outer and upper end of each strand. Collateral bundle surrounded by bundle sheath two to three cells deep; bundle sheath in transverse section broadly heart-shaped. Some cells exterior to bundle sheath differentiated into transfusion elements, which are more numerous laterally than above or below bundle sheath. Mesophyll not differentiated into palisade cells, lacunar in region of furrows. Stomata, confined to furrows, in regular linear rows.

Sigillariopsis cordata has been found in very few of the coal balls investigated, but when it does occur it is abundantly represented and is remarkably well preserved. Usually not all the tissues are equally well preserved in one leaf nor at any one level, but in the assemblage of leaves it is possible to work out the structure in considerable detail. Figure 9 shows the position and relation of several tissues in transverse section. Save for the bundle sheath, it appears extraordinarily like *S. halifaxensis* Graham, from the Halifax Hard Beds, Lower Coal Measures, Great Britain (5). The cellular detail, however, reveals structural features not in accord with the description of *S. halifaxensis*.

The xylem is organized in two median parallel strands, each composed of nine to



FIGS. 9–20.—Figs. 9–12, *Sigillariopsis cordata:* Fig. 9, diagram of transaction. Noé coll. H 2. Fig. 10, detail of portion of vascular region showing two xylem strands. Fig. 11, epidermis and hypodermis from A——A of fig. 9. Fig. 12, surface view of epidermis from stomatal furrow showing stomata. Figs. 13–20, *Stigmaria ficoides:* Fig. 13, diagram of transection: *p*, pith; x^1, primary wood; x^2, secondary wood; *pr*, principal ray; *rt*, root trace; *ph*, phloem; *co*, cortex. Noé coll. H 204. Figs. 14–17, portions from different regions of transection: *ca*, cambium; *r*, wood ray. Fig. 18, from radial section. Fig. 19, from tangential section showing portion of principal ray and rootlet trace. Fig. 20, rootlet trace with secondary wood.

fifteen elements, with smaller elements at the outer and upper ends (fig. 10). The surrounding parenchyma is for the most part irregularly and poorly preserved, but that below the xylem is more extensively developed, is irregular in size and shape of cells, and appears to be the region of the phloem. The bundle sheath is a broadly heart-shaped band of thick-walled cells, one to three cells deep (figs. 9, 10). Exterior to the bundle sheath are the transfusion elements with delicate reticulated to scalariform markings on their walls (fig. 10*tr*). The transfusion tissue does not form a complete zone about the vascular strand; but as in *Lepidophyllum*, there are more thickened elements at either side of the vascular strand than above or below it.

The hypodermis is a striking feature of this leaf. It is thickest about the abaxial keel, thinnest in the region of the adaxial median groove, and absent about the stomatiferous furrows. The hypodermis is composed of tangentially elongated cells which are roughly isodiametric in transverse section (fig. 11). The lumina of the cells are often filled with some dark material, and this in combination with the extremely thick walls renders the hypodermal layer almost opaque.

Stomata were found only about the lateral abaxial furrows. A surface view of the epidermis about the furrows shows a regular and unique stomatal pattern. The cells are arranged in longitudinal rows; the stomatal rows, consisting of guard cells alternating with short and broad epidermal cells, are separated by two rows of long and narrow epidermal cells (fig. 12).

II. Stigmaria and stigmarian rootlets

Stigmaria ficoides Br.

In Carboniferous floras there is probably no genus more widely distributed or more abundantly represented than is *Stigmaria* and its appendages, stigmarian rootlets. Fossils of *Stigmaria* are found with structure preserved in coal balls, and they are found as casts and impressions in the shales interbedded with the coal. Yet for the number of specimens and the variety of methods of preservation, there is great uniformity in the superficial features as well as in the anatomical structure. Comparatively few species, not more than a dozen, based upon external characters of form and size have been recorded, and only three or four species established upon knowledge of internal anatomical structure. Of the specimens in the latter category, by far the greater number fall into the species *S. ficoides*. When the variation in the superficial characters is considered, however, and also the fact that *Stigmaria* is the basal or rhizomatous portion of the many species—more than 150—of *Lepidodendron*, *Sigillaria*, and *Bothrodendron*, it would seem that among the many specimens there should be some expression of specific difference in anatomical detail thus far not discovered. Even so, in the following description

there seems no justification for regarding the specimen as other than *S. ficoides*, as it is diagnosed.

Stigmaria ficoides has been described from American coal fields (**6**), but the writer has not given the locality nor the horizon from whence it came other than the Pennsylvanian of Illinois. While describing his specimen as *S. ficoides*, HOSKINS (**6**) makes this reservation: "recognizing the probability that the rather large variation of forms now included in this species may eventually be separated specifically."

The fragment here considered is a piece of wood about 15 cm. long by 2.5–3 cm. in diameter. On one side the phloem and a portion of the cortex is intact. The fragment was so largely composed of pyrite that the thin sections were opaque, but the structural features were discernible in reflected light. A diagram of the transverse section shows the extent of the preservation (fig. 13). The central cavity is encircled by a narrow zone of pith, beyond which is the primary wood, limited in amount and with the smallest elements toward the center (fig. 14*px*). Secondary wood makes up most of the fragment. The tracheids with scalariform markings on both radial and tangential walls are disposed in regular radial rows (figs. 14, 15, 18, 19), with one to seven rows separated by uniseriate rays one to six cells deep (figs. 15, 16). The wood, both primary and secondary, in transverse section has the appearance of organization into discrete wedge-shaped bundles separated by broad parenchymatous rays which SCOTT (**19**) has termed the "principal rays." In tangential section the principal rays are seen as lenticular masses in spiral succession. These masses of tissue are quite ten times as long as wide, which is in contrast with previously described specimens where the length is scarcely twice that of the width (**6, 19**). The thin-walled, radially elongated cells of the ray (fig. 17) are subtended by the spiral and scalariform tracheids of the rootlet trace (fig. 19 *rt*). In the lower part of the rootlet trace the tracheids are continuous with and merge into those of the vascular cylinder.

This specimen, unlike most, has retained some of the softer and more fragile tissues beyond the secondary wood (figs. 13, 15, 16, 18). The preservation is poor and the tissues incomplete; nevertheless there is enough to show a cambial region of relatively small, regularly arranged, thin-walled cells, succeeded by phloem of larger and irregular cells. The phloem in turn is bounded by the inner cortex, a region (if one may rely on the integrity of the preservation) composed of alternating layers of tangentially elongated cells and isodiametric cells of larger caliber (fig. 16). The inner cortex is incomplete and there is no preservation beyond it.

STIGMARIAN ROOTLETS.—Almost everyone who has worked with coal ball material has commented upon the abundance and frequency of stigmarian rootlets. They are found not only intermingled with other vegetable debris, but they penetrate tissues as the pith region of stems of any and all genera of contemporary plants.

Rootlet traces in the stele and inner cortex have secondary wood (figs. 15, 20). Occasionally a free rootlet is encountered with secondary wood, but most of them have primary wood only. Usually only the thick-walled cells, which are those of the outer cortex and xylem, are preserved; but some are found with sufficient preservation to demonstrate a central position of the triangular monarch stele, while others demonstrate just as clearly an eccentric position (fig. 21 A, B).

III. Reproductive structures

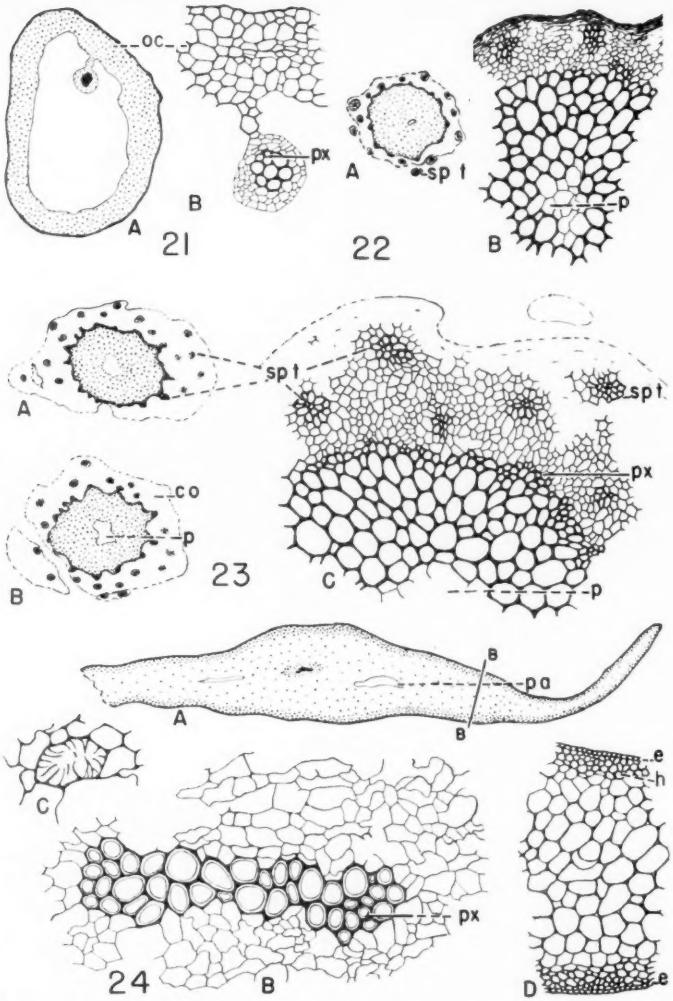
Lepidostrobus, axis of strobilus

There were two fragments of cone axes in coal ball H 227, and while the two may have originated from the same strobilus, yet the variation in size and the relative proportion of the several tissues indicate an origin from separate strobili. The smaller axis measures about 1.7 mm. in diameter and the larger about 2 mm. The larger fragment is also longer and shows some diminution in size at successive levels, which is in accord with a previously described *Lepidostrobus* axis (3).

In both specimens there is a central pith. It is limited to some fifteen thin-walled parenchymatous cells in the smaller axis (fig. 22 B), while in the larger it is more extensive but the only parenchyma cells remaining are the few disintegrating ones about the periphery of the central cavity (fig. 23 C). Surrounding the pith is the relatively broad zone of primary wood organized with the larger elements toward the center, and the smaller, protoxylem, arranged in fifteen to eighteen peripheral clusters projecting from the surface of the woody cylinder (figs. 22 A; 23 A, B). The xylem is surrounded by a zone of thinner-walled tissue, the diameter of which approximates or exceeds that of the wood (figs. 22 B; 23 C). In this region it is not possible to delimit phloem from cortex, but it is assumed that the innermost cells are those of the phloem while the outermost are doubtless cortical. Imbedded in this tissue are the sporophyll traces (figs. 22 B; 23 C, *sp t*). At any one level these are seen in different stages in their passage from the protoxylem points, where they have their origin, through the cortex. Such a distribution of traces indicates not only spiral succession, but also that they pursued an oblique course. The sporophyll trace bundles are concentric.

Lepidostrobus, cone scale

Perhaps the most complete description of a *Lepidostrobus* cone scale is Chrysler's (4), based upon material from the Lower Coal Measures of Great Britain; but Chrysler neither applies a specific name nor does he feel convinced that the scale belongs to the genus *Lepidodendron*, as the name *Lepidostrobus* implies. Although the cone scales here considered differ from those described by Chrysler in certain specific details, yet for these too a species name is withheld, inasmuch as it seems possible and probable that the scale or sporophyll will be found attached to



FIGS. 21–24.—Fig. 21, *Stigmaria ficoides*, rootlet. A, diagram showing excentric position of stele: *oc*, outer cortex. B, detail of stele and portion of outer cortex: *px*, protoxylem. Figs. 22, 23, *Lepidostrobus*, axis. Fig. 22 A, diagram of transection: *sp t*, sporophyll trace. B, portion from pith to periphery of 22 A. Fig. 23, A and B the same axis at different levels. C, detail from pith to periphery of A. Fig. 24, *Lepidostrobus*, cone scale or sporophyll. A, diagram of transection: *pa*, parichnos. B, vascular strand of A. C, transfusion element from A. D, portion of transection from B——B of A: *e*, epidermis; *h*, hypodermis. Noé coll. H 227.

a sporangium, and if so, a specific name now applied might be rendered superfluous.

The shape of the scale in section and the distribution of the tissues are shown in figure 24. The scale is not only essentially foliaceous, but—aside from the size—bears a strong resemblance to *Lepidophyllum*. The longest fragment measured was about 6 cm. This does not represent the entire length, for the basal end was not preserved. The lower end measures 17 mm. wide by 2 mm. thick. From these dimensions it tapers gradually to an acuminate tip, but still remains relatively thick, being about 0.7 mm. thick where it has diminished to 3 mm. in width.

The xylem of the central vascular strand consists of some forty elements arranged in a horizontally elongated band, with the smaller elements at either end (fig. 24 B). There is some indication that phloem surrounds the xylem, but—as in *Lepidophyllum*—there is a greater display of irregular thin-walled cells on the abaxial side. Unlike *Lepidophyllum* or *Sigillariopsis*, there is no encircling sheath of fibrous cells; but like those foliar organs, there are some transfusion elements lateral and adjacent to the vascular strand (fig. 24 C). At either side of the vascular strand, and separated from it by the width of the strand itself, are tangentially elongated lacunar areas comparable in position and appearance with the parichnos of the leaf bases of *Lepidodendron* and so labeled here (fig. 24 A). In this cone scale the two parichnos strands parallel the vascular strand to within 1 cm. of the tip. The mesophyll is differentiated into an inner zone of large cells (roughly circular in transverse section) and small intercellular spaces and an outer more compact zone, four to five cells deep (fig. 24 D). The cells of the outer zone are of smaller caliber and have slightly thicker walls. The epidermis is a uniform layer of small, relatively thick-walled cells. No stomata were observed.

In its known dimensions and general appearance the cone scales here recorded resemble those described by CHRYSLER (**4**). The variation is in the organization of the conducting elements and adjacent tissue. The bundle of the latter, with its surrounding cells which form a U-shaped pattern on the abaxial side, is reminiscent of the vascular strand of *Lepidophyllum thomasi*, while the bundle of the former more nearly resembles that of *L. alatum* or *L. latifolium*.

Although these cone scales are five to ten times as wide as any of the species of *Lepidophyllum* just described, yet it is not size that precludes their inclusion among foliar organs of *Lepidodendron*, for many of the species of *Lepidophyllum*, based upon impressions, are as large or larger (**9, 11**). It is rather their structure, in harmony with that of the pedicel subtending the sporangium, that establishes these organs as the distal laminal portion of the sporophyll of *Lepidostrobus;* and while organic connection is lacking, yet the evidence—that of association and histological similarity—indicates relationship with *Lepidocarpon*.

Lepidostrobus, microsporangium and spores

This description is based on a single and isolated specimen consisting of the pedicel of a sporophyll bearing on its adaxial face a sporangium in which are tetrads of small spores. Despite its being a sole specimen, it possesses several features that are unique, distinctive, and specific.

The pedicel is incompletely preserved, but its general shape is outlined in figure 25 A–G (a series of diagrams of tangential sections from the proximal to the distal region of the sporangium). Proximally the pedicel is narrow, with a sharp abaxial keel, but it becomes progressively broader and flatter, with the laminal margins curving about the base of the sporangium. The xylem of the single, median, concentric vascular strand is composed of some thirty elements, arranged in a horizontally elongated to elliptical mass (fig. 26 B). As in the *Lepidostrobus* cone scale, there is no sheath of fibrous cells about the vascular strand.

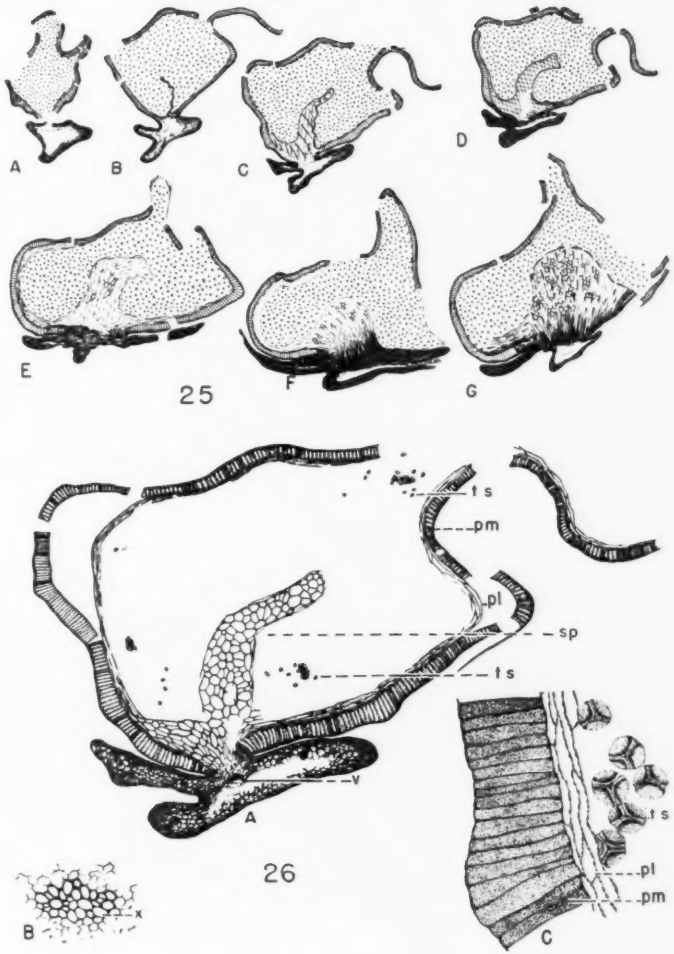
The dimensions of the sporangium, 10 by 2 by 2 mm., are only approximate, for it has been partially crushed; moreover, a small but indeterminable portion of the radial dimension—radial with respect to the axis of the strobilus—was lost in sectioning. The wall of the sporangium is composed of two layers: an outer unicellular and conspicuous layer of radially elongated, thick-walled cells and an inner layer, three cells deep, of thin-walled cells, elongated at right angles to those of the former layer (fig. 26 C). There were no recognizable tapetal cells.

A specific feature of this sporangium is the structure and extensive development of the subarchesporial pad. In the tangential section nearest the axis of the cone there is no indication of sterile tissue within the sporangium (fig. 25 A). In the next section there is a thin band of sterile tissue extending from the base of the sporangium into the sporangial cavity for a distance of one-third the diameter of the sporangium (fig. 25 B). In succeeding tangential sections the tissue becomes broader and thicker, forming in the distal region a massive dome of sterile tissue (fig. 25 G). The cells composing the subarchesporial pad are relatively thin-walled, isodiametric, and large, with an average diameter of 60–70 μ.

The sporangial cavity is filled with innumerable immature spores which are not separated from one another but are in the tetrahedral formation as they emerged from the spore mother cell. The tetrad is about 30 μ in diameter. At this stage the spores are free from superficial ornamentation (fig. 26 C).

Lepidostrobus, megaspores (*Trilete*s Reinsch emend, Schopf)

It is rare that a sectioned coal ball will not yield isolated megaspores; among the megaspores there is considerable variation in size and configuration of spore coat, but thus far they have been consistently alike in the amount of preservation, being represented in almost every instance by the thick impervious spore coat only. When an occasional megaspore has been found with some content (fig. 29), it has



FIGS. 25, 26.—*Lepidostrobus*, microsporangium. Fig. 25, diagrams of tangential sections from proximal to distal region of sporangium. Noé coll. H 227. Fig. 26 A, tangential section of microsporangium: *pm*, prismatic layer of wall of sporangium; *pl*, parietal layers of same; *ts*, tetrads of spores; *sp*, subarchesporial pad; *v*, vascular strand. B, xylem strand of A. C, detail of wall layers and tetrads of spores from A.

been valueless in making further contribution to the development and organization of the megagametophyte, for the content is noncellular and so indistinct that it is impossible to tell whether it is an integral part of the megaspore or some intruded material. The few megaspores selected for description here fall within the dimensions and proportions of *Triletes*, an established genus for megaspores of Paleozoic lycopods (**16**). While they demonstrate variation in size, shape, ornamentation, and thickness of spore coat to the extent of specific differences, yet because they are not known in all their dimensions and in their surface detail, they have not been assigned species names; instead they are recorded as "types."

Type I.—This is not only the largest of the megaspores, excepting that of *Lepidocarpon*, but it also has the thickest spore coat. It is an almost spherical body with an average inside diameter of about 850 μ. The spore coat is about 85 μ thick. Both megaspores of figure 27 give evidence of a pronounced equatorial flange and apical suture; otherwise the surface is smooth. The only indication of multiple layers of the spore coat is seen in figure 27 A, where a layer (designated the endospore) is partially separated and infolded.

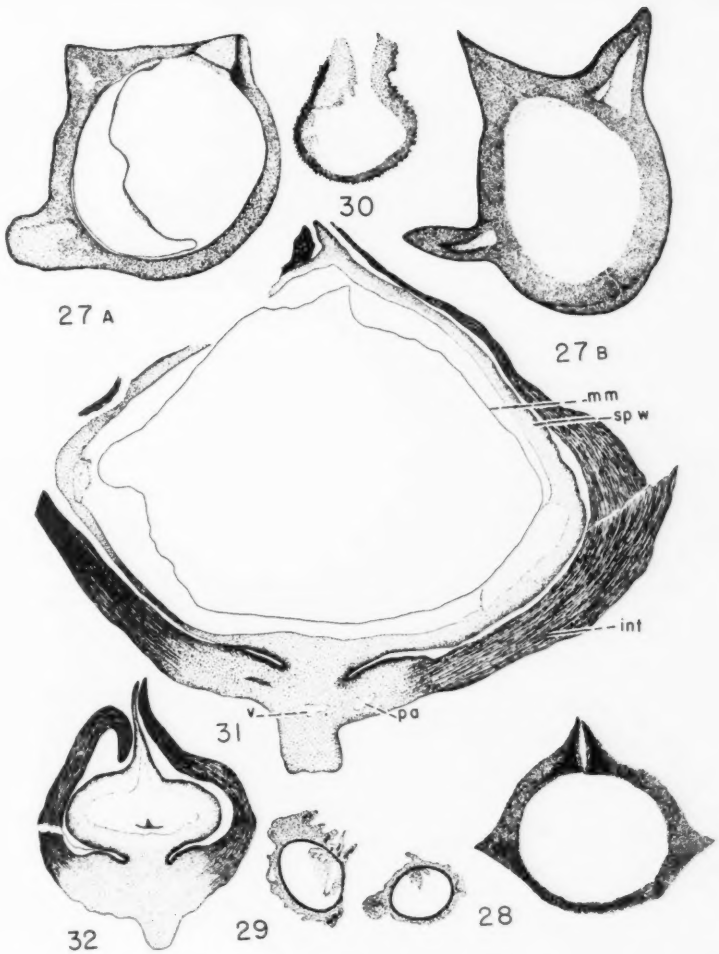
Type II.—The spore body is slightly broader than long, with an equatorial diameter of 607 μ and an axial diameter of 514 μ. The spore coat averages about 60 μ thick (fig. 28). The spore coat is not so thick as is that of the preceding type, and both apical suture and equatorial flange are much less pronounced. There is no observable differentiation into multiple layers.

Type III.—The spore body has an equatorial diameter of 480 μ and an axial diameter of 360 μ; these are inside dimensions, exclusive of the spore coat. The spore coat is differentiated into two distinguishable layers: an inner layer, that appears to be homologous with the endospore of the spores of modern pteridophytes, and averages about 20 μ thick; an outer layer, perispore, that is wrinkled and folded, and variable in thickness from places so thin as to be scarcely visible to 150 μ. Within the spore is some vague and indistinct content, as already mentioned (fig. 29).

Type IV.—This spore is longer than broad, with an axial diameter of 537 μ and an equatorial diameter of 450 μ. The spore coat averages about 30 μ thick. There is no equatorial flange nor uneven and thick perispore, as in the preceding types, instead—in so far as may be judged from a section of the spore—the surface was uniformly and rather finely reticulated (fig. 30).

Lepidocarpon lomaxi Scott (**17**)

In an earlier paper (**12**) some young unintegumented specimens of *Lepidocarpon* were described. Subsequent investigation has revealed other specimens of such forms as well as those with integuments fully developed. Some of the specimens in the former category possess certain details of preservation that were lacking in



FIGS. 27–32.—Figs. 27–30, *Triletes:* Fig. 27, A and B, Type I. Noé coll. H 239. Fig. 28, Type II. Noé coll. H 227. Fig. 29, Type III. Noé coll. H 216. Fig. 30, Type IV. Noé coll. H. 236. Figs. 31, 32, *Lepidocarpon lomaxi*. Fig. 31, diagram of median tangential section: *mm*, megaspore membrane; *sp w*, wall of sporangium; *int*, integument; *v*, region of vascular strand; *pa*, parichnos. Fig. 32, another integumented specimen of *Lepidocarpon* drawn to same scale as that of fig. 31.

the previous description and so provide supplementary information; whereas in the latter category are specimens which not only show the structure of the mature sporangium and its inclosing integument, but also serve to sustain if not to substantiate some postulated theories with regard to the nutrition of the megagametophyte.

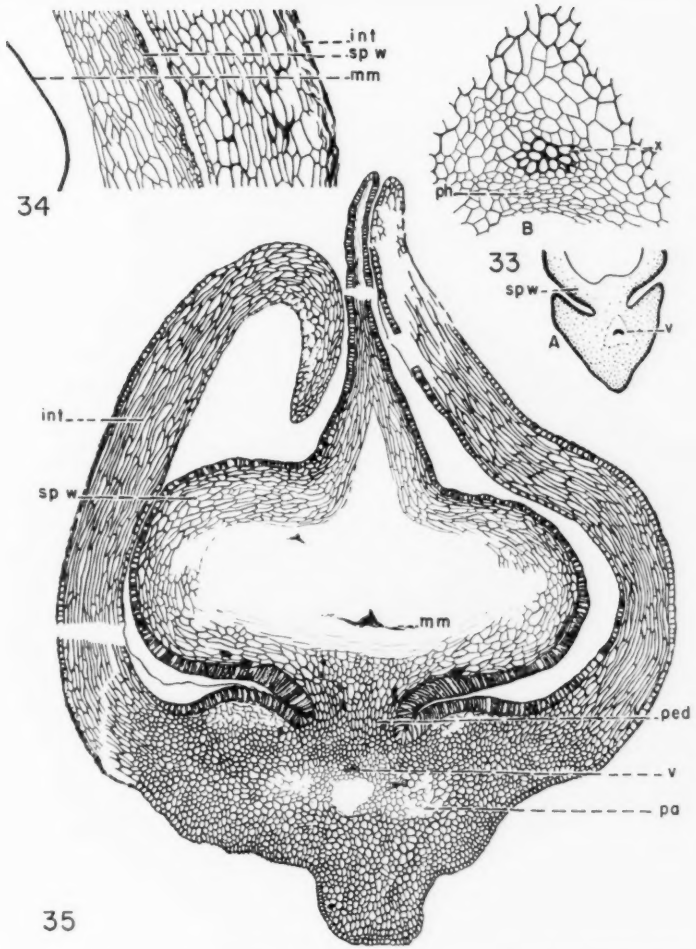
In the previous account some of the specimens indicated the position of the vascular strand of the pedicel, but in none of them was it sufficiently preserved to show the organization of the vascular elements. Figure 33 A is a diagram of the basal portion of tangential section of *Lepidocarpon* showing the position of the vascular strand and its relation to the tissues of the pedicel and sporangium, and a detail from A (fig. 33 B) shows the cellular organization of the xylem and phloem. The vascular strand resembles that of some of the species of *Lepidophyllum*, particularly *L. alatum* and *L. latifolium;* but—as in the cone scale and sporophyll pedicel of *Lepidostrobus*—it is not inclosed by a sheath of thick-walled fibrous cells.

Mature specimens of *Lepidocarpon* are recognized by the presence of the so-called "integument." The early stages in the formation of the integument have not been observed, but from the available material certain facts with respect to its origin and development may be deduced. That it was not formed until the sporangium had attained, or almost attained, maximum size is evident from the dimensions of some of the young unintegumented specimens. That it had its origin in the lateral margins of the pedicel of the sporophyll is indicated by a comparison of the relative position of the tissues, vascular strand and parichnos, in the young and old specimens.

The integument is a multicellular layer thicker than the wall of the sporangium (figs. 34, 35). It is composed of some fifteen to eighteen rows of vertically elongated cells bounded by an epidermis of smaller and isodiametric cells. There is no trace of any vascular tissue, nor are there any sclerenchymatous cells. The integument, therefore, would not be a very rigid layer.

Figure 34 is a detail of a portion of the megaspore membrane, the wall of the sporangium, and the integument, showing their relation to one another. A comparison of the megaspore membrane and the sporangium wall of this integumented specimen with those of a young sporangium (**12**, fig. 9) reveals for the older sporangium a thinner megaspore membrane but a much thicker sporangial wall. These differences demonstrate a progressive evolution and elaboration of the tissues surrounding the megaspore that is not terminated with its maturation but persists with the development of the megagametophyte and the formation of the integument.

Among the many specimens of *Lepidocarpon* found in coal ball H 227 are two unusually well preserved integumented ones, drawn to the same scale in figures 31



FIGS. 33–35. *Lepidocarpon lomaxi:* Fig. 33 A, diagram of basal portion of tangential section of immature *Lepidocarpon* showing position of vascular strand. B, detail of vascular strand of A. Fig. 34, detail of portion of megaspore membrane, wall of sporangium, and integument from mature specimen. Fig. 35, median tangential section of the *Lepidocarpon* shown diagrammatically in fig. 32. Noé coll. H 227 B 12.

and 32. The dimensions of height and width of the two, respectively, are 9 by 11 mm. and 3.5 by 3 mm. Such a discrepancy in size of specimens of apparently the same age would make it difficult to reduce them to the same species, were it not for another feature that suggests an alternative explanation. The megaspore membrane of the larger is normal in position and thickness for the integumented stage in the ontogeny of *Lepidocarpon;* in the smaller there is a remnant of the membrane in the basal region of the sporangium (fig. 35*mm*), as if there had been an incipient development of the megaspore and consequent abortion of the megagametophyte. It is suggestive of an unpollinated ovule in a strobilus of *Zamia;* in such an ovule there is cessation of development, with final disintegration of the megagametophyte or endosperm, and at the same time continuous but less extensive development of the surrounding sporophyte tissues, nucellus, and integument.

Summary

1. These studies were based on coal ball material collected by Dr. A. C. Noé of the University of Chicago. The coal balls were found near Harrisburg, Illinois, in coal no. 5, in the Alleghany group of the Upper Pennsylvanian.

2. The genera described are both vegetative and reproductive organs belonging in the Lepidodendrales order.

3. The genera possessing specific diagnostic features either are harmonized with established species or are described as new; those represented by a single specimen only, or those with insufficient preservation, and therefore inadequately known, are described and recorded under the generic name.

4. The genera and species described are: *Lepidophyllum aciculum* sp. nov.; *L. trichosulcata* sp. nov.; *L. alatum* sp. nov.; *L. latifolium* Graham; *L. thomasi* Graham; *Sigillariopsis cordata* sp. nov.; *Stigmaria ficoides* Br., and stigmarian rootlets; *Lepidostrobus* Br. cone axis, cone scale, and microsporangium with tetrads of spores; *Triletes* Reinsch (megaspores of *Lepidodendron*), four types; *Lepidocarpon lomaxi* Scott.

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LITERATURE CITED

1. Bertrand, C. E., Remarques sur le *Lepidodendron harcourtii* de Witham. Trav. Mém. Facultés de Lille, no. 2. 1891.
2. Calder, Mary G., Notes on the Kidston collection of fossil plants. Phil. Trans. Roy. Soc. Edinburgh **58**:49–62. 1934.
3. Campos, J. D., A new specimen of *Lepidostrobus foliaceus*. Bot. Gaz. **79**:441–449. 1925.
4. Chrysler, M. A., On the structure of the cone scales of *Lepidostrobus*. New Phytol. **35**: 267–274. 1936.

5. GRAHAM, ROY, An anatomical study of the leaves of the Carboniferous aborescent lycopods. Ann. Bot. **49**:587–608. 1935.
6. HOSKINS, J. H., Notes on the structure of Pennsylvanian plants. BOT. GAZ. **85**:74–82. 1928.
7. KOOPMANS, R. G., Researches on the flora of the coal balls from the "Finefrau-Nebenbank" horizon of the Province of Limburg (The Netherlands). 1928.
8. KRICK, HARRIETTE V., Structure of seedlike fructifications found in coal balls from Harrisburg, Illinois. BOT. GAZ. **93**:151–172. 1932.
9. LESQUEREUX, LEO, Coal flora of Pennsylvania. **2**: 1880.
10. LINDLEY, JOHN, and HUTTON, WILLIAM, The fossil flora of Great Britain. **2**: London. 1835.
11. NOÉ, A. C., Pennsylvanian flora of Northern Illinois. Illinois State Geol. Surv. Bull. 52. 1925.
12. REED, FREDDA D., *Lepidocarpon* sporangia from the Upper Carboniferous of Illinois. BOT. GAZ. **98**:307–316. 1936.
13. ———, Notes on some plant remains from the Carboniferous of Illinois. BOT. GAZ. **100**: 324–335. 1938.
14. ———, Structure of some Carboniferous seeds from American coal fields. BOT. GAZ. **100**: 769–787. 1938.
15. RENAULT, M. B., Structure comparée de quelques tiges de la Flora Carbonifère. Nouv. Arch. Mus. D'Historie Naturelle **2**:214–348. 1870.
16. SCHOPF, J. M., Spores from the Herrin (no. 6) coal bed in Illinois. Ill. State Geol. Surv. Report of Investigation, no. 50. 1938.
17. SCOTT, D. H., On the structure and affinities of fossil plants from the Paleozoic rocks. IV. The seed-like fructifications of *Lepidocarpon*, a genus of Lycopodiaceous cones from the Carboniferous formation. Phil. Trans. Roy. Soc. London B. **194**:291–335. 1901.
18. ———, On the occurrence of *Sigillariopsis* in the Lower Coal Measures of Britain. Ann. Bot. **18**:519–521. 1904.
19. ———, Studies in fossil botany. Part I. London. 1920.
20. SEWARD, A. C., Notes on the Binney collection of Coal Measure plants. I. *Lepidophlois*. Proc. Camb. Phil. Soc. **10**:137–156. 1899.

DEVELOPMENT OF SEEDLINGS OF HETEROPOGON CONTORTUS AS RELATED TO SOIL MOISTURE AND COMPETITION

GEORGE E. GLENDENING

(WITH FOUR FIGURES)

Introduction

On the grazing ranges of southwestern United States many important forage grasses reproduce from seed only during exceptionally favorable years, although there may continually be a supply of viable seed in the soil. Germination normally occurs during July or August, as a result of soil moistening by sporadic rains characteristic at that time. High temperatures and low relative humidity lead to rapid drying of surface soils; consequently conditions favorable to germination and seedling growth occur only during intermittent short periods.

This study of seedling development of a typical range grass, tanglehead (*Heteropogon contortus* (L.) Beauv.), was made under range conditions during the summer of 1938 and in a greenhouse during the winter of 1938 and spring of 1939. Tanglehead is a coarse, perennial bunchgrass of wide distribution (**5**). In the United States it is of local importance in the warmer and drier parts of Texas, New Mexico, and Arizona. The field study was designed to determine the rate and manner of shoot and root development under natural soil and climatic conditions, while the objectives of the greenhouse study were to determine, under partially controlled conditions, the effects of soil moisture and of competition upon the development of shoots and roots and survival or death of the seedlings during periods of drought.

Other studies (**1, 7, 16**) have shown that later reactions of grasses, including resistance to drought and winter injury, are closely associated with the amount of growth, particularly root development, made during seedling stages. Soil moisture has been shown (**19, 20, 21**) to affect top and root growth of grasses, with maximum root development occurring under moisture conditions below the optimum for top growth. It has also been shown (**17, 20**) that extent of root development in grasses varies with soil texture, greatest penetration and widest spread occurring in loose soils as compared with that in compact soils. Investigations (**11, 13, 14, 15, 20**) have indicated that the effects of competition among plants for various factors of the habitat, with respect to reduction of top and root growth, are consistent and subject to quantitative measurement.

Methods of study

In the field

The field study plot was located on the Santa Rita Experimental Range, south of Tucson, Arizona, at an altitude of 3000 feet. The vegetation, representing a transition from desert plains grassland to southern desert shrub, is composed mainly of trees and shrubs, including *Prosopis velutina*, *Acacia greggii*, *Aplopappus fruticosus*, *Calliandra eriophylla*, *Krameria glandulosa*, and others. Numerous artificial revegetation tests have indicated that this area represents approximately the lower range of perennial grass stands. At present such grasses as *Bouteloua rothrockii*, *B. chondrosioides*, *B. filiformis*, *B. curtipendula*, *Aristida divaricata*, *A. glabrata*, *Hilaria belangeri*, *Trichachne californica*, *Triodia mutica*, *T. pulchella*, and others occur locally on the better sites.

The soil on this area has been classified as Continental gravelly loam (**22**). At the surface it is a sandy loam with a small amount of red clay. At approximately 12 inches the clay content increases, and a layer of loose small stones is encountered. Samples from the 6-inch level have a moisture equivalent of 6.587 per cent and a wilting coefficient, as determined indirectly (**2**), of 3.58 per cent.

High summer temperatures, low relative humidity, and distinctly seasonal rainfall are characteristic. Records taken at the area during the 5-year period preceding this study show that mean maximum air temperatures varied from 98° F. in July to 61° F. in January, while the mean minima for these two months were 72° and 34° F., respectively. Summer temperatures frequently exceed 100° F., while freezing temperatures occur only occasionally during the winter months. Monthly mean maximum soil temperatures (measured at the 6-inch level) varied from 102° F. in June to 49° F. in January, while the minima were 84° and 38° F., respectively, for the same months. Maximum surface soil temperatures of 150° F. have been recorded on this area. During the 5-year period, the average mean maximum wet-bulb depression for the months of June, July, August, and September was 30° F., and the total annual evaporation from a free-water surface (shallow tank) was in excess of 100 inches, or approximately eight times the total annual precipitation of 12.40 inches. There are two distinct rainfall seasons and two dry periods. Approximately 60 per cent of the total annual rainfall occurs during the months of July, August, and September, and about 25 per cent falls during December, January, and February. A spring dry period occurs characteristically during April, May, and June, and the month of October is usually dry. Climatic records taken during July, August, and September of 1938 indicate that conditions for plant growth were fairly typical during the course of the study, but the season was a little cooler and drier than the average for the preceding 5-year period.

On July 12, 1938, a plot 8×16 feet, within a cattle and rabbit exclosure, was

planted with tanglehead seed. As a result of two periods of favorable rainfall, crops of seedlings emerged on July 23 and August 6. At 2- to 4-day intervals, seedlings from the two crops were excavated, and the following observations and measurements taken: number of leaves and length of longest leaf; length of primary root and of its laterals; number and length of adventitious roots and length of lateral roots from them. Altogether, 101 seedlings were examined during a 49-day period. At each excavation, samples for soil-moisture determinations were taken at the surface-inch, 6-inch, and 12-inch level. Moisture determinations were made by standard desiccation methods.

In the greenhouse

This phase, conducted at the University of Arizona, included the observation and measurement of tanglehead seedlings grown in prepared soil in pots under four varying amounts of moisture and three different intensities of competition.

On November 11, 1938, seed was planted in ninety-three no. 2 glazed pots in a homogeneous soil composed of two parts of clean river sand and one part of silt loam, the mixture having a moisture equivalent of 6.197 per cent. On November 21 the pots were divided into a W series and an S series for treatment under varying amounts of moisture and different intensities of competition, respectively. The W series was further divided into four groups, WA, WB, WC, and WD, of thirteen pots each, watered at intervals of 10, 4, and 2 days, and at irregular intervals, respectively. Each pot contained ten seedlings. The S series was divided into three groups, SA, SB, and SC, of thirteen pots each, with twenty, ten, and four seedlings per pot, respectively. These pots were watered at 4-day intervals. All pots received one-half pint of water at each irrigation.

The location of the pots on the greenhouse bench and the sequence of examination were randomized (**18**). The plants were grown for 6 weeks, during which the WA, WB, WC, and WD pots received the equivalent of 1.4, 2.9, 5.7, and 2.6 surface inches of water, respectively, while all pots under the competitive treatments (S series) received 2.9 inches.

Beginning November 27, 1938, and at twice-weekly intervals until January 2, 1939, the plants in one pot under each treatment were washed out for observation. Just prior to this removal of the plants, samples of soil for moisture determinations were obtained.

On January 2, irrigation of the remaining three pots under each treatment was discontinued. On February 14, the plants in one pot from each treatment were washed out to determine the growth made before drought dormancy set in, and on the same day irrigation of a second pot from each treatment was begun to determine whether or not the plants would resume growth. The third set of plants,

one pot from each treatment, was allowed further to dry out until March 17, when water was applied.

In addition to observations made in the field study, dry weights of shoots and roots were determined, and photographs of representative plants were obtained at critical periods.

Air temperature in the greenhouse was regulated thermostatically and was further controlled by manipulation of ventilators. Usually temperatures were lower and relative humidity greater than on the field plot, while sunlight intensity and length of day were less. On the whole the character of growth made by the seedlings was similar to that on the field plot.

Results

In the field

Growth data for the two crops of seedlings are summarized in table 1, and drawings of representative plants are shown in figures 1 and 2. Growth was slow at first and mainly confined to elongation of the primary root. When the first leaves emerged, the primary root was slightly over 1 inch in length and directed vertically downward. It continued to elongate vertically for approximately 30 days. The primary root system consists of only the primary root with its laterals; no other seminal roots are developed. The maximum length measured was 10.4 inches, but the greatest average length of the primary roots of any group of seedlings was 6.4 inches. Lateral roots appeared after the third day and reached an average length of about 1 inch at 18–20 days. Most of these were formed in a zone 2–3 inches below the soil surface. After 20–30 days the cortex was invariably sloughing on the primary roots, the laterals were dry and fragile, and the growing points had apparently ceased to elongate.

Adventitious or nodal roots appeared 15 and 23 days after emergence on the July 23 and August 6 plants, respectively, but were organized somewhat earlier since plants left in water over night on the twelfth day developed short adventitious roots. A comparison of the data in tables 1 and 2 shows that in each crop of seedlings adventitious roots were first recorded a few days after rainfall had resulted in available moisture in the surface soil.

Tillering seemed definitely associated with development of adventitious roots. This agrees with the work of others (**6, 19**). Leaf number increased slowly at first and then rather rapidly following development of nodal roots. At the close of the study most plants had two or three tillers and a few of the largest had four.

In the greenhouse

Variation in moisture.—Table 3 shows that frequency of watering was reflected directly in moisture content and relative wetness (**3**) of the soil in the pots.

Average relative wetness of the soil was 84, 111, 168, and 111 in the WA, WB, WC, and WD pots from November 27 to January 2. An obvious difference resulting from this variation in moisture supply was that of number and length of leaves.

TABLE 1

GROWTH MEASUREMENTS (IN INCHES) ON FIELD-GROWN SEEDLINGS

Date	No. of days after emergence	Leaves		Primary root system		Adventitious root system		
		Number	Length of longest	Length of primary root	Length of laterals	No. of roots	Length of roots	Length of laterals
		Crop of July 23, 1938						
July 24	1	1	0.3	1.2				
26	3	2	0.3	1.6	Trace			
28	5	2	0.3	2.7	0.1			
August 1	9	3	0.4	3.2	0.5			
4	12	3	0.5	3.2	0.5			
7	15	4	0.7	3.5	0.7	1	Trace	
11	19	6	0.9	4.3	0.8	2	0.1	
16	24	6	1.5	.....	.....	2	0.5	
23	31	11	2.1	.....	.....	3	2.2	1.0
26	34	14	2.5	.....	.....	4	6.4	1.0
September 7	46	24	3.7	.....	.....	6	4.1	3.0
		Crop of August 6, 1938						
August 7	1	1	0.3	1.2				
11	5	2	0.4	1.9	0.2			
14	8	3	0.5	2.5	0.4			
16	10	4	0.4	2.7	0.5			
19	13	5	0.6	3.4	0.6			
24	18	7	1.3	5.3	1.0			
29	23	14	1.3	6.4	.....	Trace	Trace	
September 1	26	17	1.7	.....	.....	4	1.8	0.5
4	29	17	1.9	.....	.....	4	3.7	1.0
7	32	19	3.2	.....	.....	4	4.0	1.0
11	36	22	2.8	.....	.....	5	4.8	3.0

This difference was small at first (table 4) but increased with age. On January 2, 44 days after emergence, both length and number of leaves were directly proportional to frequency of irrigation.

The rate of elongation of the primary roots was about equal under all treatments for approximately 30 days. The primary roots in the WC and WD plants elongated very little after December 20, however, while those of the WA and WB

plants were still growing on January 2. Maximum lengths attained were 15.11, 15.47, 12.31, and 13.40 inches in the order WA to WD. Lateral roots were similar in length in the WA, WB, and WD plants but were more numerous on the latter two than on the least watered (WA) plants. They were most numerous and of greatest length on the most watered (WC) plants.

Adventitious roots appeared on the WC plants on December 4, 15 days after emergence, and on the WB and WD plants 19–23 days after emergence (table 5). Development of adventitious roots in the WA plants was meager. Adventitious

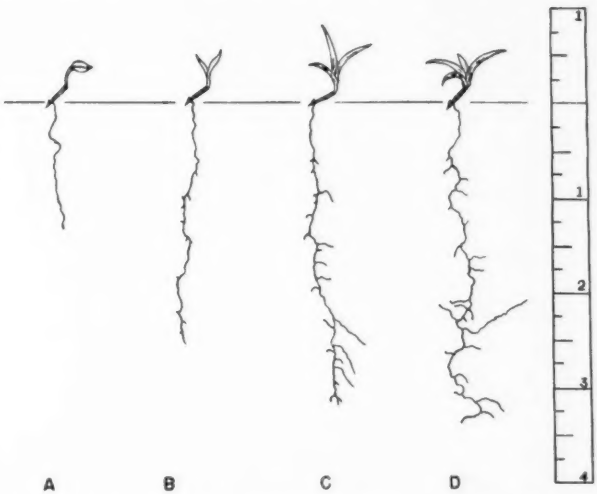


FIG. 1.—Early stages in development of tanglehead seedlings on field plot. Plants *A* to *D* excavated 1, 5, 9, and 12 days, respectively, after emergence of shoots.

root laterals were few to entirely absent on the WA and most numerous on the WC plants. Sequence and extent of tillering varied directly with frequency of watering. Representative plants from each of the treatments are shown in figure 3.

The dry weights of shoots on January 2 were 0.034, 0.056, 0.090, and 0.074 gm. per plant, and those of roots (total) 0.02, 0.038, 0.036, and 0.041 gm. under the WA, WB, WC, and WD treatments, respectively. These show that weight of shoots was directly proportional to irrigation frequency, and that root weights followed a similar but less definite trend. Shoot/root ratios on January 2 were 1.70:1, 1.47:1, 2.50:1, and 1.80:1 in the WA, WB, WC, and WD plants.

COMPETITION.—The effect of variation in number of plants per pot upon seedling development was less pronounced than that of variation in frequency of watering (table 6). For approximately 30 days leaf growth varied directly with

denseness of planting. By January 2, however, this condition was reversed and the lengths were 5, 5.30, and 5.86 inches in seedlings grown at the rate of twenty, ten,

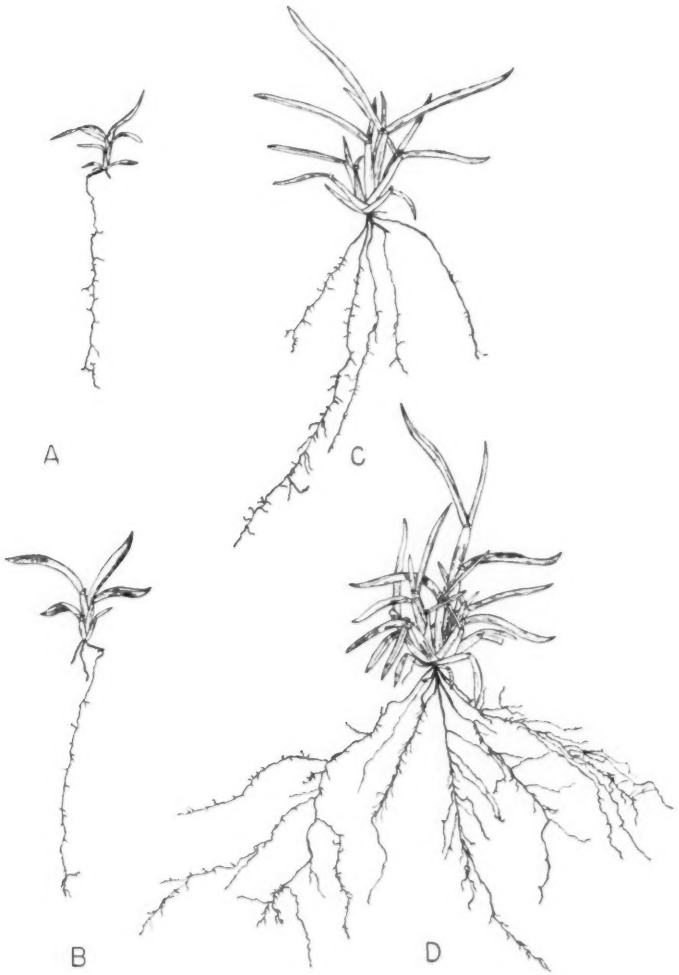


FIG. 2.—Later stages in growth of seedlings on field plot. Plants *A* to *D* excavated 19, 24, 34, to 45 days after emergence. Plant *D* is slightly above average in size.

and four plants per pot. Density of planting had no significant effect on the number of leaves and amount of tillering.

On January 2, lengths of the primary roots were 12.27, 15.47, and 14.13 inches

in the SA, SB, and SC pots. Primary root laterals were fewest in the SA plants and mainly confined to the lower portions of the root. They were more numerous and more generally distributed in the SB and SC plants. Adventitious roots appeared

TABLE 2

RAINFALL, PERCENTAGE SOIL MOISTURE, AND RELATIVE WETNESS* ON FIELD PLOT

Date		Rainfall (inches)	Surface inch		6-inch level		12-inch level	
			Soil m. (%)	Rel. wet.	Soil m. (%)	Rel. wet.	Soil m. (%)	Rel. wet.
July	11	0.01						
	17	0.15						
	20	1.03						
	21		6.8	103	7.1	108	6.1	92
	22	0.02						
	23	0.04						
	24		3.3	50	6.0	91	6.2	94
	26		0.7	11	5.4	82	5.5	83
	28		0.4	6	4.8	73		
August	1		0.2	3	3.7	56	4.9	74
	3	0.17						
	4	0.51	0.8	12	3.1	47	4.6	70
	6	2.02						
	7		8.0	121	10.0	152	10.1	153
	9		4.1	62	6.8	103	8.3	126
	11		0.7	11	5.7	86		
	12	0.98						
	14		6.3	95	8.2	124	8.6	130
	16		1.4	21	6.9	104	7.1	108
	19		0.02	0.3	5.3	80	4.4	67
	24	0.14	5.0	76	3.8	58	4.7	71
	25	0.14						
	26		1.0	15	4.5	68	5.1	77
	28	0.04						
	29		0.4	6	4.9	74	5.2	79
	30	0.30						
September	1		0.8	12	4.9	74	4.8	73
	2	0.20						
	4		2.3	35	4.9	74	4.9	74
	5	0.37						
	7		1.6	24	6.5	98	6.3	95
	11		0.3	5	5.1	77	5.2	79

* Percentage soil moisture divided by moisture equivalent, the quotient ×100, as suggested by CONRAD and VEIHMEYER (3).

after about 3 weeks, and in all cases rapid elongation of the primary root system appeared to cease.

Adventitious roots were most numerous under the medium dense spacing (SB) and fewest on the plants (SA) grown under the greatest competition (table 7). The rate of elongation was similar under all degrees of competition. On January 2, lengths of adventitious roots (8.66, 8.33, and 8.15 inches) varied directly with denseness of planting, while lengths of adventitious laterals (1.11, 1.75, and 1.92

inches) were inversely related to degree of competition. Representative plants photographed on January 2 are shown in figure 4.

TABLE 3

PERCENTAGE SOIL MOISTURE AND RELATIVE WETNESS IN POTS UNDER VARIOUS TREATMENTS DURING PERIOD OF IRRIGATION

DATE	WA		SB-WB*		WC		WD		SA		SC	
	SOIL M. (%)	REL. WET.	SOIL M. (%)	REL. WET.	SOIL M. (%)	REL. WET.	SOIL M. (%)	REL. WET.	SOIL M. (%)	REL. WET.	SOIL M. (%)	REL. WET.
Nov. 27	6.3	102	5.7	92	9.0	145	6.9	111	5.2	84	4.6	74
30	4.6	74	6.6	106	6.0	97	7.2	116	7.8	126	6.9	111
Dec. 1	5.7	92	7.2	116	9.1	147	7.9	127	6.6	106	4.9	79
4	4.7	76	7.8	126	10.2	165	7.9	127	6.1	98	6.9	111
8	6.8	110	6.9	111	11.8	190	5.5	89	4.5	73	4.9	79
10	4.8	77	7.8	126	8.0	129	6.3	102	4.3	69	6.7	108
12	5.5	89	5.6	90	7.7	124	7.3	118	6.5	105	7.7	124
15	7.3†	118	6.1	98	8.5	137	7.8	126	6.4	103	8.4	135
20	5.5	89	8.0	129	11.4	184	8.6	139	7.0	113	6.9	111
23	5.3	85	7.2	116	14.7	237	6.3	102	7.2	116	6.8	110
27	4.7	76	7.2	116	14.4	232	6.0	97	7.6	123	7.9	127
30	3.9	63	7.6	123	10.2	165	5.6	90	6.0	97	5.4	87
Jan. 2	4.4	71	5.7	92	14.4	232	5.9	95	5.4	87	5.8	94
Mean	5.2	84	6.9	111	10.4	168	6.9	111	6.2	100	6.5	105
High	6.8	110	8.0	129	14.7	237	8.6	139	7.8	126	8.4	135
Low	3.9	63	5.6	90	6.0	97	5.5	89	4.3	69	4.6	74

* Average of two pots. † Watered by mistake and not included in average.

TABLE 4

GROWTH OF LEAVES AND PRIMARY ROOT SYSTEM AS AFFECTED BY FREQUENCY OF WATERING

DATE*	AVERAGE LENGTH (INCHES)											
	WA			WB			WC			WD		
	LEAVES	PRIMARY ROOT	LATERALS	LEAVES	PRIMARY ROOT	LATERALS	LEAVES	PRIMARY ROOT	LATERALS	LEAVES	PRIMARY ROOT	LATERALS
November 30–December 1	1.90	5.70	1.75	2.38	4.79	1.62	2.18	5.25	1.50	2.74	7.93	2.00
December 8–12†	3.01	9.24	2.84	3.45	10.72	2.45	3.11	9.62	3.29	2.61	9.61	2.72
December 15–20	3.97	11.31	.....	4.20	11.07	.....	5.97	12.07	.....	5.14	12.66	.....
December 23–27	4.00	14.64	.....	4.45	15.01	.....	8.03	12.31	.....	6.15	13.20	.....
December 30–January 2	3.90	15.11	.....	5.30	15.47	.....	7.07	11.55	.....	6.06	13.40	.....
No. of leaves January 2	(4.4)			(6.6)			(8.0)			(6.8)		

* Figures represent average of measurements on the two dates shown.
† No measurements of lateral roots made after this date.

On January 2 the average dry weight of roots per plant was 0.027, 0.038, and 0.041 gm. under the SA, SB, and SC treatments, while shoot weights were 0.051, 0.056, and 0.057 gm., showing that the weight of shoots and roots was inversely

related to the degree of competition. Shoot/root ratios in the same order were 1.88:1, 1.47:1, and 1.39:1, indicating greatest relative shoot production under the most intense competition.

FINAL GROWTH AFTER DISCONTINUED WATERING.—The moisture in all the pots soon after watering ceased was below the wilting coefficient (3.37 per cent) in all

TABLE 5

DEVELOPMENT OF ADVENTITIOUS ROOT SYSTEM AS AFFECTED BY FREQUENCY OF WATERING

DATE*	NO. OF ROOTS				LENGTH OF ROOTS (INCHES)				LENGTH OF LATERAL ROOTS (INCHES)			
	WA	WB	WC	WD	WA	WB	WC	WD	WA	WB	WC	WD
November 30–December 4	.....	.....	0.80	.....	.....	.....	1.32	.....	.....	.....	.....	.....
December 8–12	.....	0.30	1.00	0.30	.....	1.69	1.98	0.25	.....	.....	.....	.....
December 15–20	0.55	1.65	1.40	1.60	1.05	1.74	6.57	4.70	.....	0.35	0.98	0.44
December 23–27	0.40	1.70	2.65	1.40	0.23	6.21	6.12	8.01	.....	0.76	2.87	1.61
December 30–January 2	0.40	2.05	2.60	1.80	2.85	8.33	8.34	10.48	0.15	1.75	4.63	1.26

* Figures represent average of measurements on the two dates shown.

TABLE 6

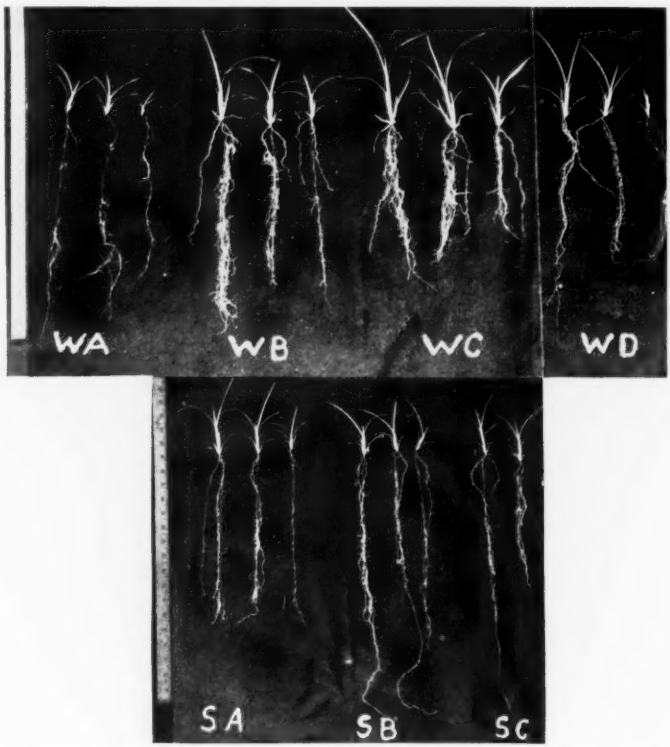
GROWTH OF LEAVES AND PRIMARY ROOT SYSTEM AS AFFECTED BY COMPETITION

DATE*	AVERAGE LENGTH (INCHES)								
	SA			SB			SC		
	LEAVES	PRIMARY ROOT	LATERALS	LEAVES	PRIMARY ROOT	LATERALS	LEAVES	PRIMARY ROOT	LATERALS
November 30–December 4	2.52	5.66	1.87	2.38	4.79	1.48	2.16	7.13	0.81
December 8–12†	3.06	11.85	2.55	3.45	10.72	2.45	2.96	10.68	3.62
December 15–20	4.77	10.65	.....	4.26	11.67	.....	3.98	11.14	.....
December 23–27	4.90	12.21	.....	4.45	15.01	.....	4.46	13.26	.....
December 30–January 2	5.00	12.27	.....	5.30	15.47	.....	5.86	14.13	.....
No. of leaves January 2	(5.3)			(6.6)			(5.7)		

* Figures represent average of measurements on the two dates shown.
† No measurements of laterals made after this date.

samples taken after January 25, indicating that a serious moisture shortage existed from January 25 to February 14. At the end of the 6-week dry period all the plants were completely dormant, with those in the most frequently watered (WC) and least densely planted (SC) pots appearing to be drier than those grown with less moisture and under more intense competition. One set of plants on February 14 showed that leaf growth made before dormancy set in was slight in the WA and

WD, somewhat greater in the WB, and much greater in the WC plants. Under all treatments, with the exception of the SC plants, where the number of leaves tripled, the number of leaves on February 14 was approximately double that on January 2.



FIGS. 3, 4.—Fig. 3 (above), largest-, smallest-, and average-sized plants from pots watered at 10-, 4-, 2-day, and irregular intervals, in order from left to right. Fig. 4 (below), representative seedlings from pots receiving equal amounts of water but containing 20, 10, and 4 plants, respectively, from left to right. Top growth of SA plants is above average in size, but root growth is typical.

Primary root systems, with the exception of those of the WA plants which had elongated to 16.75 inches, were badly disintegrated, and no measurements other than weight were made. With few exceptions the number, length, and length of laterals of the adventitious roots on the plants examined 6 weeks after irrigation ceased were directly proportional to the frequency at which the plants had previously been watered and inversely proportional to the number per pot. The final dry weights of shoots and roots showed the same relationship. Shoot/root ratios

were not consistent as affected by moisture but tended to increase with the amount of water applied, while with respect to competition the shoot/root ratios were inversely proportional to the number of plants per pot.

Resumption of growth by the plants when watered after the dry-up period of 6 weeks was rapid. Most plants showed some greening of leaves within 24 hours and on the second day were again growing rapidly. New growth was initiated from the axial buds of the basal leaves, and in most cases the old leaves became active. Under all treatments at least some of the old leaves regained their turgor and became green. Flowering occurred in the WD plants only 7 days after watering was resumed. The remaining plants then flowered in the order SB, WB, SC, WC, SA,

TABLE 7

DEVELOPMENT OF ADVENTITIOUS ROOT SYSTEM AS AFFECTED BY COMPETITION

DATE*	NO. OF ROOTS			LENGTH OF ROOTS (INCHES)			LENGTH OF LATERAL ROOTS (INCHES)		
	SA	SB	SC	SA	SB	SC	SA	SB	SC
November 30–December 4	.....	.....	.....	.....	.....	.....	.....	.....	.....
December 8–12	0.10	0.30	0.25	1.52	1.69	3.05	.....	.....	.....
December 15–20	1.30	1.65	1.40	3.48	1.74	1.90	0.43	0.40	1.20
December 23–27	1.55	1.70	1.50	7.09	6.21	7.62	0.84	1.16	0.90
December 30–January 2	1.35	2.05	2.00	8.66	8.33	8.15	1.11	1.75	1.92

* Figures represent average of measurements on the two dates shown.

and WA, with the last plants in the anthesis stage on March 22, 36 days after watering was resumed.

As compared with the recovery at the close of the 6-week dry period, resumption of growth after 11 weeks of drought was slow, although there was noticeable greening of old leaves in the SC plants within 24 hours after being watered, and by the fourth day there were new leaves from basal buds on the WA, WD, SB, and WB plants. There was no sign of renewed growth in the SA and WC plants, and examination showed them to be dead. In the plants which had been grown under the least water (WA and WD), many of the old leaves became green.

Discussion

The rate of growth of the field-grown seedlings during the first 40 days after emergence was relatively slow, although soil moisture at the 6-inch level was above the wilting coefficient and therefore, according to CONRAD and VEIHMEYER (3), sufficient to produce normal growth at all times. The relatively slow growth rate of perennial grasses has been pointed out by PARKER and SAMPSON (12). Despite

their small size at the end of the experiments, the seedlings remained alive during the fall dry period which followed.

Data from both the range and the greenhouse indicate that moisture content of the soil is probably the major controlling factor in the development of these plants under range conditions. Depth of penetration of primary roots and the zone of maximum lateral root development correspond closely with the depth to which water from the periodic showers penetrates.

Under field conditions the initiation of adventitious or permanent roots is directly controlled by surface soil moisture. This was also the case in the greenhouse, where plants grown in pots in which the surface soil was usually dry developed an average of less than one adventitious root per plant during 6 weeks of growth. LOCKE and CLARK (10) noted a similar condition, wherein wheat grown in Oklahoma under severe drought conditions did not develop adventitious roots.

In all cases where the tanglehead seedlings developed adventitious roots, the primary roots soon ceased to elongate and became very brittle. There is some disagreement in the literature as to the function of primary roots in grasses after the adventitious root system is established. HAYWARD (4) suggests that the primary roots of corn are of little importance after the adventitious root system is established, and LA RUE (9) indicates the same with regard to wheat, barley, and other grasses. KRASSOVSKY (8), however, states that the seminal roots of summer wheat, barley, and rye remain functional up to the time of harvest. It was impossible to determine definitely whether or not the primary roots of the tanglehead seedlings ceased to function after the adventitious roots were established, but it is significant that in plants where few or no adventitious roots developed, the primary roots remained intact and definitely elongated up to the final set of observations.

The relatively meager development of shoots as compared with roots in tanglehead seedlings is probably significant. In general the ratio of shoots to roots increased in direct proportion to the amount of water applied, which is in agreement with the results of other studies (15, 19). These ratios, based on air-dry weight, varied from 1.7:1 to 2.5:1 and are very low compared with those reported for Sudan grass (11), which is considered a relatively drought-resistant species. The value of a large absorbing system coupled with a small transpiring surface in plants, which under natural conditions are frequently subjected to adverse moisture conditions, is hardly to be questioned.

Denseness of planting appears to be of minor importance during the early stages of seedling development and while soil moisture is available. When moisture becomes limiting, thickly planted seedlings are at a definite disadvantage as compared with those growing under less crowded conditions and having a lower total moisture requirement per unit of soil. Seedlings planted at the rate of

twenty per pot failed to resume growth after a drought period of 11 weeks, whereas those grown at the rate of four per pot became green within 24 hours after being watered, and those planted at the rate of ten per pot became green after 48 hours. Similar responses have been noted on artificially seeded field plots, where densely planted seedlings appeared to make growth comparable with that of those planted less densely during the first growing season while moisture was available but suffered much greater drought losses during subsequent dry periods.

The fact that the old leaves of some plants regained their turgor and became active within 24 hours after water was applied following drought periods of 3 and 8 weeks, during which soil moisture was below the wilting coefficient, shows that the protoplasm of these leaves had not been permanently injured by the extremely unfavorable moisture conditions and is indicative of the inherent drought resistance of tanglehead seedlings.

Summary

1. Based on dry weight, maximum development of shoots and roots of tanglehead grass seedlings occurred under most frequent irrigation and least intense competition. Shoot/root ratios were greatest under most frequent watering and most intense competition.

2. Lack of moisture at the soil surface retarded the initiation of adventitious roots under field and greenhouse conditions.

3. Tillering was closely associated with the establishment of the adventitious root system.

4. Soil moisture had a greater effect upon the plants than competition and appears to be the major controlling factor under range conditions.

5. Rapid resumption of growth after drought periods of 3 and 8 weeks, during which soil moisture was below the wilting coefficient, indicates the inherent drought resistance of these seedlings.

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LITERATURE CITED

1. Aamodt, O. S., and Johnston, W. H., Studies on drought resistance in spring wheat. Canad. Jour. Res. **14**:122–152. 1936.
2. Briggs, L. J., and Shantz, H. L., The wilting coefficient for different plants and its indirect determination. U.S. Dept. Agr. B.P.I. Bull. 230. 1912.

3. CONRAD, J. P., and VEIHMEYER, F. J., Root development and soil moisture. Hilgardia **4**: 113–134. 1929.
4. HAYWARD, H. E., The structure of economic plants. New York. 1938.
5. HITCHCOCK, A. S., Manual of the grasses of the United States. U.S. Dept. Agr. Misc. Publ. 200. 1935.
6. JACKSON, V. G., Anatomical structure of the roots of barley. Ann. Bot. **36**:21–39. 1922.
7. KEIM, F. D., and BEADLE, G. W., Relation of time of seeding to root development and winter survival of fall-seeded grasses and legumes. Ecology **8**:251–264. 1927.
8. KRASSOVSKY, I., Physiological activity of the seminal and nodal roots of crop plants. Soil Sci. **21**:307–325. 1926.
9. LA RUE, C. D., Regeneration in monocotyledonous seedlings. Amer. Jour. Bot. **22**:486–492. 1935.
10. LOCKE, L. F., and CLARK, J. A., Development of wheat plants from seminal roots. Jour. Amer. Soc. Agron. **16**:261–268. 1924.
11. NEDROW, W. W., Studies on the ecology of roots. Ecology **18**:27–52. 1937.
12. PARKER, K. W., and SAMPSON, A. W., Growth and yield of certain Gramineae as influenced by reduction of photosynthetic tissue. Hilgardia **5**:361–381. 1931.
13. PAVLYCHENKO, T. K., Quantitative studies of entire root systems of weeds and crop plants under field conditions. Ecology **18**:62–70. 1937.
14. PAVLYCHENKO, T. K., and HARRINGTON, J. B., Competitive efficiency of weeds and cereal crops. Canad. Jour. Res. **10**:77–94. 1934.
15. PERALTA, F. DE, Some principles of competition as illustrated by Sudan grass, *Holcus sorghum sudanensis*. Ecol. Monog. **5**:355–404. 1935.
16. SHULTZ, H. K., and HAYES, H. K., Artificial drought tests of some hay and pature grasses and legumes in sod and seedling stages of growth. Jour. Amer. Soc. Agron. **30**:676–682. 1938.
17. SINGH, K., Development of root system of wheat of different kinds of watering. Ann. Bot. **36**:353–360. 1922.
18. SNEDECOR, G. W., Statistical methods. Collegiate Press, Ames, Iowa. 1937.
19. WEAVER, J. E., Root development of field crops. New York. 1926.
20. WEAVER, J. E., and CLEMENTS, F. E., Plant ecology. New York. 1929.
21. WELTON, F. A., and WILSON, J. D., Water supplying power of the soil under different species of grass and with different rates of water applications. Plant Physiol. **6**:485–493. 1931.
22. YOUNGS, F. O., SWEET, A. T., STRAHORN, A. T., GLASSEY, T. W., and POULSON, E. N., Soil survey of the Tucson area, Arizona. U.S. Bur. Chem. and Soils, Soil Survey Rept. 19. 1936.

MEGAGAMETOPHYTE DEVELOPMENT IN A TRIPLOID TULIP[1]

JOHN M. BELLOWS, JR., AND RONALD BAMFORD

(WITH FORTY-THREE FIGURES)

Introduction

An earlier study (**6**) of the progeny resulting from reciprocal crosses of triploid ($2n = 36$) and diploid ($2n = 24$) tulips showed that the chromosome number of the functional gametes of the triploid is less than the expected number, if normal meiotic divisions, like those of the diploids, had taken place. The formation of the male gametes in triploids has been dealt with extensively (**7, 8, 9, 13, 17, 25, 34, 38, 42, 48**). The general conclusion seems to be that the functional gametes fail to receive the expected number of chromosomes, especially in the case of autotriploids, because of the chromosome association in the meiotic prophases. Separation of the chromosomes results in an unequal distribution, bridging and eventual fragmentation, lagging, and finally the loss of chromosomes during the meiotic or subsequent divisions. In addition to this there may also be a selection due to differential pollen-tube development and later to certain types of incompatibility. All these types of behavior explain the selection in favor of the diploid gametes, and in certain cases the tetraploid gametes (**6, 26, 27, 29, 40, 46**), which must function when the triploid is used as the pollen parent.

In contrast to the volume of publications concerning pollen formation in the triploid, there has been much less work on the meiotic and subsequent divisions during the formation of the megagametophyte in triploids. The most complete accounts of this development have been those of MORINAGA and FUKUSHIMA (**30**) on *Oryza*, SATINA and BLAKESLEE (**39**) on *Datura*, and WESTFALL (**47**) on *Lilium*. These all report the unequal and irregular distribution of chromosomes so frequently reported for meiosis in pollen formation. However, when the progeny of crosses involving the triploid as a seed parent are examined (**6, 9, 17, 20, 23, 26, 27, 29, 32, 39, 46**), they clearly indicate that a somewhat different type of behavior must occur during megagametophyte formation, because the female gametes usually have a higher chromosome content. DARLINGTON (**16**) has previously pointed this out. Our earlier studies (**6**) of $3n \times 2n$ crosses, as compared with $2n \times 3n$, have shown that Inglescombe Yellow followed the behavior mentioned, and it is the purpose of this paper to discuss the relation the megagametophyte divisions have to this difference between the triploid as a pollen and as a seed parent.

[1] Contribution no. 713, Maryland Agricultural Experiment Station.

The type of megagametophyte division in diploid tulips has been described by BAMBACIONI and others (**3, 4, 12, 18, 19**), and there is little doubt that it falls in the category more recently described by COOPER and others (**14, 15, 37, 47**) for several species of *Lilium*. Extensive reviews concerning this type have been made by JOSHI (**22**) and SCHNARF (**41**). The latter has applied the term *Fritillaria*-type to such megagametophytes.

Material and methods

Inglescombe Yellow, the triploid tulip selected for this study, is one of the Leiostemones group. UPCOTT (**44**) considers it a variety of *Tulipa gesneriana*, and —although it has proved to be self-sterile—it has been reciprocally crossed with certain diploids (**6, 44, 49**).

Collections of unopened buds from Maryland-grown bulbs were made in the field in the spring of 1938 and 1939. Additional collections of opened buds were made in the spring of 1940 from plants grown in the greenhouse. It was found that the final stages in the development of the megagametophyte do not take place until the flower has opened and the anthers are dehiscing. This study bears out the report of UPCOTT and LA COUR (**45**), however, that meiosis in the female gamete takes place just before the flower bud opens.

Fixations were made in Navashin's fluid. The material was treated with the butyl-alcohol technique and imbedded in Tissuemat. Sections were cut at 18–20 μ and stained with Heidenhain's iron-alum haematoxylin. Examination of the section was made with 16, 4, and 1.2-mm. apochromatic objectives and 15× compensating oculars.

Observations

The basic chromosome number in *Tulipa* is 12. Inglescombe Yellow, an autotriploid horticultural variety, was found to contain 36 chromosomes in the somatic cells of the early nucellar layer and in the surrounding cells of the ovule and ovary.

The megagametophyte arises by differentiation from a subepidermal cell which develops directly into the megaspore mother cell without undergoing further division. The megaspore mother cell increases in size until it is two to three times as long as wide. The nucleus increases in size to some extent with the cell. When both have attained maximum size, prior to the prophase stage of the first division, the cytoplasm of the cell is finely granular, very dense in appearance, and no large vacuoles are present (figs. 1, 19). One or more large nucleoli are usually found, in addition to many smaller nucleoli, in each nucleus. Immediately preceding the prophase stage the large nucleus of the megaspore mother cell will occupy a position in the center of the cell or slightly toward the micropylar end.

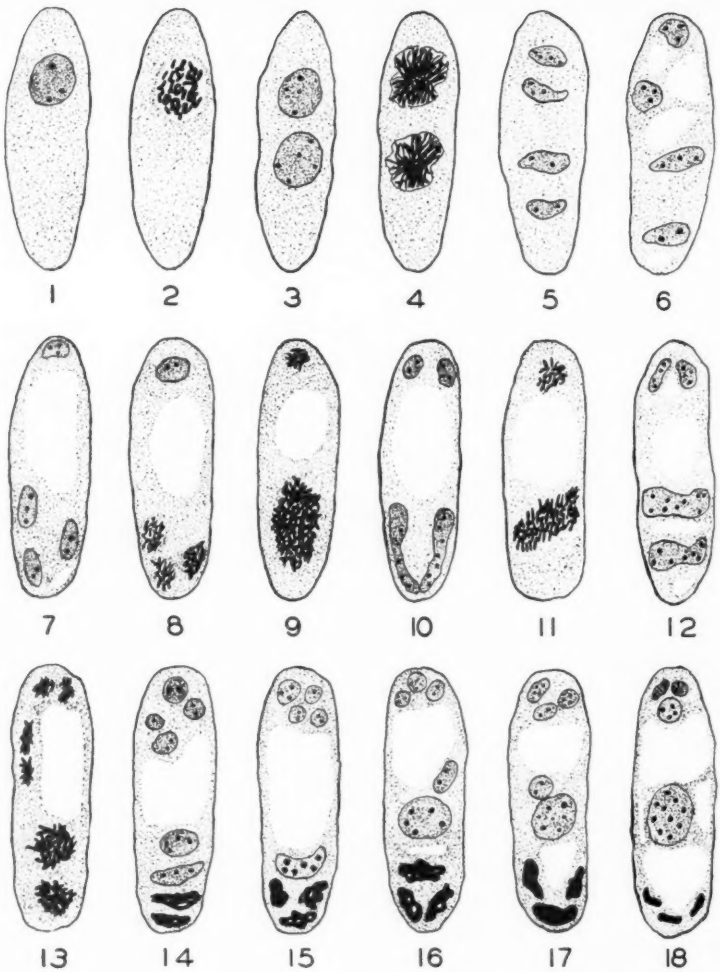
The first stage at which it was possible to determine the number and type of association among the chromosomes was at the early diakinesis of meiosis I (fig.

20). In one case a total of 6 trivalent and 9 bivalent chromosomes could be identified, but it was very difficult to obtain the total for all the cells observed. Later, during the formation of the metaphase plates for the first division, it was easier to determine the exact type of association. Again trivalents, bivalents, and univalents could always be identified (fig. 2). One metaphase plate contained 5 trivalents, 8 bivalents, and 5 univalents; another contained 6 trivalents, 6 bivalents, and 6 univalents; a third contained 6 trivalents, 5 bivalents, and 8 univalents.

Separation of the chromosomes in the anaphase stage seems to indicate that an 18–18 distribution is the most common (figs. 21, 22). Counts were made of one or both plates of this stage when possible. In one megasporocyte division a 19–17 distribution, and in another a 19–16 distribution, was observed. Only one division showed evidence of lagging chromosome material, and this was in the form of a chromosome bridge (fig. 22). As a result of this division, two nuclei with approximately the $3n/2$ number of chromosomes are formed (figs. 3, 24), and micronuclei (fig. 23) are rare.

In the second meiotic division, an equational one, the spindles usually form in the same plane as that of the preceding, but occasionally they may form across or diagonally through the developing megagametophyte (figs. 25, 26). In one case both nuclei were in the metaphase stage and each contained 18 chromosomes. In two more both nuclei were in the anaphase stage. At the micropylar end of the first there was a 20–16 distribution, and at the chalazal end there was a 20–14 distribution. The other had a 20–15 distribution at the micropylar end and a 19–17 distribution at the chalazal end. It was difficult to make exact counts, but the distribution between the nuclei in many cases was approximately equal. Infrequently micronuclei appear in the cytoplasm after this division, indicating that some chromatin material has failed to be included in the four nuclei.

This division results in the formation of four megaspore nuclei, each with approximately 18 chromosomes, which align themselves throughout the length of the megagametophyte (fig. 5). After this division one of the nuclei at the micropylar end of the sac begins to migrate toward the two nuclei at the chalazal end (figs. 6, 27, 28), and as it approaches them the three move into a triangular position. A large vacuole partially separates the three at the chalazal end from the single one at the micropylar end (figs. 7, 29). Soon each of the three nuclei passes through the prophase stage, and three metaphase plates of chromosomes can be recognized (figs. 8, 30). The chromosomes from each of these three nuclei are then grouped in one large mass resembling a single metaphase plate, much larger than any one of the previous plates (figs. 9, 11, 31). A typical equational division follows in which each chromosome group takes part so that two equally large nuclei result (figs. 12, 34). The direction of the spindle alignment of this division at the chalazal end may be through the long axis of the megagametophyte or across it (figs. 31, 33).



FIGS. 1–18.*—Fig. 1, megaspore mother nucleus, resting stage. Fig. 2, metaphase I. Fig. 3, two megaspore nuclei after meiosis I. Fig. 4, polar view of two dividing megaspore nuclei. Fig. 5, four megaspore nuclei after meiosis II. Figs. 6, 7, polarization of megagametophyte. Fig. 8, late prophase of three megaspore nuclei at chalazal end. Figs. 9, 11, third nuclear division. Figs. 10, 12, two triploid nuclei, chalazal end; two haploid nuclei, micropylar end. Fig. 13, fourth nuclear division. Figs. 14, 15, degeneration of antipodals. Fig. 16, migration of haploid nucleus. Fig. 17, formation of polar apparatus. Fig. 18, mature megagametophyte preceding fertilization. ×450.

* Diagrammatic drawings made either from photomicrographs or directly from microscope. Micropylar end of megagametophyte is pointed toward top of page in each case. "Figure incomplete" indicates that both ends of embryo sac were not included in one section.

The former arrangement appears more common. At the same time the nucleus remaining at the micropylar end also undergoes division. The spindle is generally aligned across the long axis of the megagametophyte (fig. 33) but may occasionally be formed at right angles to this plane.

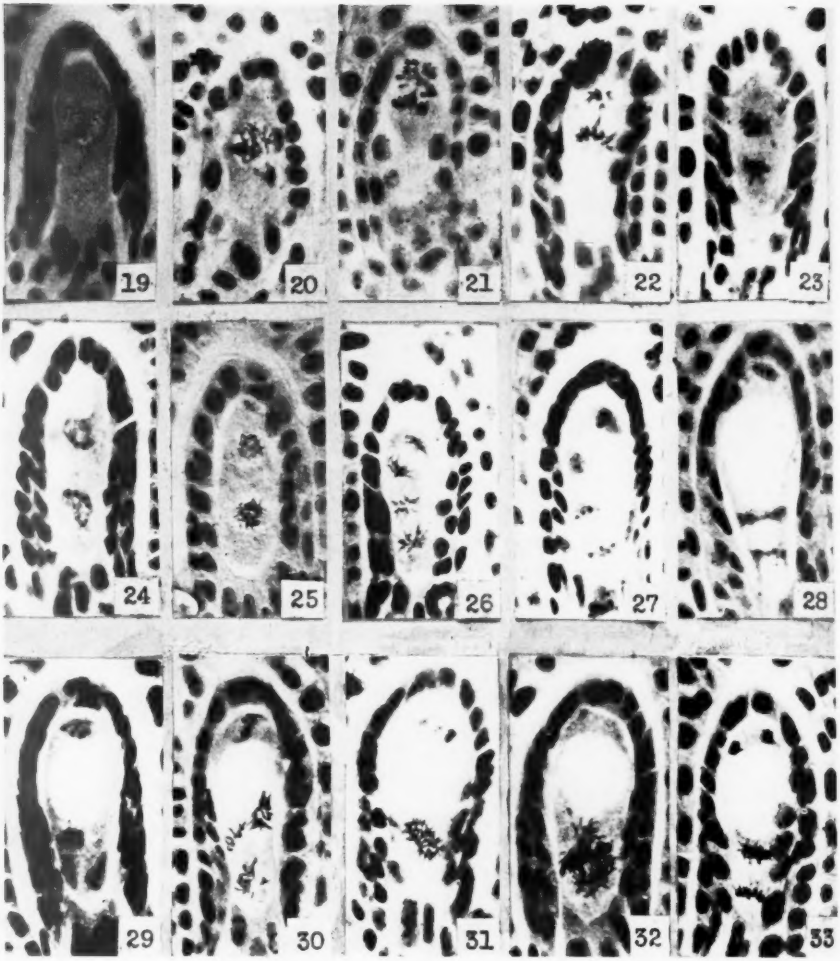
This third nuclear division is characterized, therefore, by the massing of the chromosomes from three of the megaspore nuclei on one large metaphase plate at the chalazal end of the megagametophyte. There is no actual fusion of chromatin material, but the chromosomes come together and then each divides in an equational division. The large metaphase plate would be expected to contain three times the haploid number, or approximately 54 chromosomes. The exact number would depend upon the distribution and possible loss of chromosomes in the two preceding meiotic divisions. Counts of this large metaphase plate have always been in the vicinity of 54, with a range of 51–57 chromosomes.

An anaphase stage of the division at the chalazal end in one megagametophyte gave a total of approximately 108 chromosomes between the two groups on the spindle. There is not necessarily an exact separation of 54 chromosomes to each pole. The separation is often unequal, and it is difficult to obtain exact counts of each anaphase group. As might be expected with such a large mass of chromosomes, there is some loss of chromatin matter from the nuclei, resulting in the appearance of relatively large micronuclei at completion of the division. At the micropylar end of the sac, where the remaining megaspore nucleus undergoes division, metaphase plates have given counts of 18 chromosomes, while others have had 21, 20, and 17. Lagging chromatin material was noted in only one instance (fig. 31), but no micronuclei have been observed in this division.

Thus, at the end of the third nuclear division, the megagametophyte is composed of two small nuclei at the micropylar end and two larger ones at the chalazal end. These larger ones may be arranged on the long axis of the megagametophyte (figs. 12, 34), or across it (fig. 10), depending upon the orientation of the spindle from which each arose. The large vacuole previously mentioned now completely separates the nuclei at the micropylar end from those at the chalazal end, and small vacuoles may appear elsewhere in the cytoplasm.

One megagametophyte was observed in which there were eight nuclei, all the same size; probably the fusion of three megaspore nuclei failed to take place. Instead, all four megaspore nuclei probably divided again, with the result that a megagametophyte of eight haploid nuclei was formed. The fourth and final division usually takes place at the chalazal end first. The two large nuclei first undergo division, followed by division of the two small nuclei at the micropylar end. It is not uncommon, however, for the final division to take place simultaneously in all the nuclei (figs. 13, 35).

When both nuclei participate, four large ones will result from the division, and



FIGS. 19–33.—Fig. 19, megaspore mother cell. Fig. 20, prophase I. Fig. 21, metaphase I. Fig. 22, chromosome bridge, meiosis I. Fig. 23, extruded chromatin, meiosis I. Fig. 24, two megaspore nuclei. Fig. 25, prophase II. Fig. 26, late anaphase II; figure incomplete. Fig. 27, four megaspore nuclei. Fig. 28, appearance of vacuoles. Fig. 29, polarization of megaspore nuclei. Fig. 30, metaphase, third nuclear division, micropylar and chalazal end; figure incomplete. Fig. 31, same, micropylar end; lagging chromatin at micropylar end. Fig. 32, anaphase, third nuclear division, chalazal end; figure incomplete. Fig. 33, telophase, third nuclear division, micropylar and chalazal end. ×450.

these take different positions in the chalazal end (fig. 41). Often only one of the large nuclei will undergo complete division, while the other begins to disintegrate in the prophase stage (figs. 14, 37). When this occurs only two large nuclei result, while the disintegrating nucleus is still visible. The nucleus which disintegrates is generally the one at the extreme chalazal end. Here again micronuclei are sometimes evident, showing that some chromatin material was not included in the four nuclei.

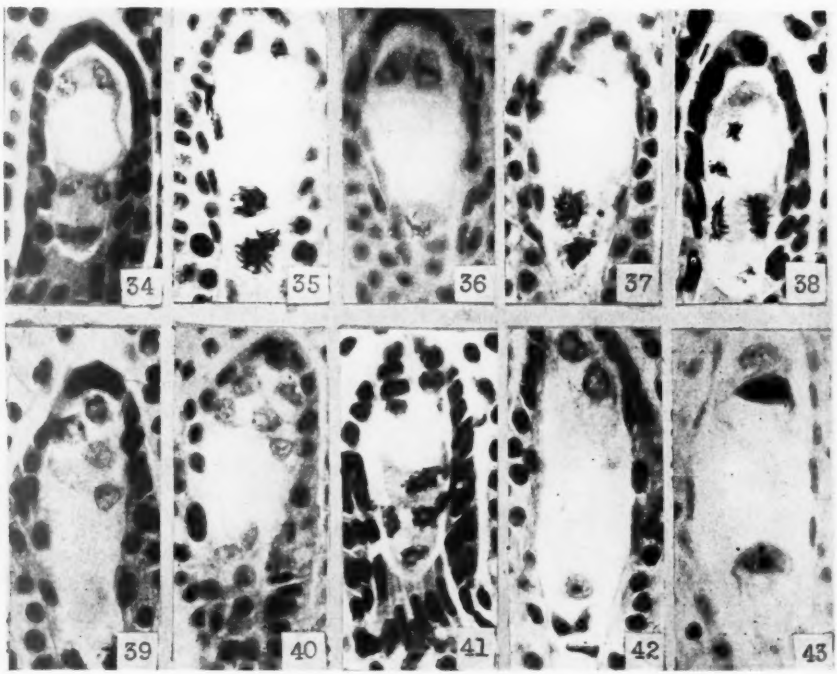
At the micropylar end, each of the two small nuclei undergo division, generally after the division is complete at the chalazal end. There seems to be no common arrangement of the spindles for this division, as they have been found oriented across the end of the megagametophyte and along either side (figs. 35, 36). In a few cases it was possible to determine the distribution of chromosomes in the anaphase stage. One had a 19–17 distribution for one nucleus and a 22–14 distribution for the other. Another showed a 20–16 distribution for one nucleus, but it was impossible to obtain an accurate count of the other. In one megagametophyte a total of 79 chromosomes was counted from the anaphase plates of the two dividing nuclei. This is an excess of 7 chromosomes over what would be expected had equal distribution taken place in the preceding divisions. The 79 chromosomes were so spread out in the cytoplasm that it was impossible positively to identify their respective nuclei. The four formed as a result of this division take different positions in the micropylar end (fig. 39).

In three rare cases five and six nuclei have been found at the micropylar end, indicating some form of irregularity in division. All the nuclei were approximately equal in size, and none appeared to be undergoing disintegration (fig. 40). The appearance of an occasional megagametophyte with five or six instead of four nuclei at the micropylar end may be brought about by failure of the scattered chromosomes to reconstitute themselves properly. No evidence of lagging or chromosome bridges was observed in this final division at the micropylar end.

At the completion of this division, the typical megagametophyte—allowing for irregular distribution and loss of chromosomes—is composed of four nuclei with approximately 18 chromosomes at the micropylar end, and two nuclei with approximately 54 chromosomes at the chalazal end. If both nuclei at the chalazal end of the sac take part in the final division, the total will be four instead of two large nuclei.

After four large nuclei have been formed at the chalazal end, the two at the extreme end first disintegrate, followed by the one next above (figs. 14, 15, 42). In some it appears that only the two at the extreme end disintegrate and the other two persist. The triploid nucleus nearest the center begins to increase in size and migrates toward the center of the megagametophyte (figs. 15, 42). One of the four small nuclei at the micropylar end also moves toward the center (figs. 16, 17).

Midway the large and the small nuclei fuse to form a polar apparatus of approximately 72 chromosomes. The three antipodals at the chalazal end are in various stages of disintegration (fig. 42); however, one of them may persist for some time. The three nuclei remaining at the micropylar end take up various positions, but one of them begins to increase in size about the time that fusion of the polar ap-



FIGS. 34–43.—Fig. 34, end of third nuclear division. Fig. 35, fourth nuclear division. Figs. 36, 37, metaphase, fourth nuclear division; note disintegrating chalazal nucleus. Fig. 38, anaphase, fourth nuclear division; figure incomplete. Fig. 39, end of fourth nuclear division, micropylar end; figure incomplete. Fig. 40, end of fourth nuclear division, micropylar end; note five nuclei; figure incomplete. Fig. 41, end of fourth nuclear division, chalazal end; figure incomplete. Fig. 42, megagametophyte showing three disintegrating antipodal nuclei, polar nucleus from chalazal end, polar nucleus at micropylar end and egg nucleus. Fig. 43, endosperm nucleus and egg nucleus. ×450.

paratus is completed (fig. 43). Generally this is the nucleus nearest the center, and it becomes the egg cell. The two remaining synergids, and any extra nuclei that may have been formed through previous irregularities in division, begin to disintegrate, although one or more sometimes persist. Degeneration of the synergids and the antipodals presumably does not interfere with development of the egg and polar nuclei.

The typical megagametophyte, at the stage preceding fertilization, now consists of a large polar apparatus in the center and an egg nucleus at the micropylar end (fig. 43). The egg nucleus increases in size, while the polar nucleus appears to decrease considerably in the final stages of development. The megagametophyte is much vacuolated, especially between the nuclei and the surrounding nucellar layer. Finally a cell wall appears around each nucleus.

Discussion

Formation of the *Fritillaria*-type (**41**) of megagametophyte has been somewhat incompletely described since the work of TREUB and MELLINK (**43**) in 1880 until COOPER's (**14, 15**) accounts in 1934 and 1935. The earlier workers (**1, 2, 3, 4, 5, 10, 11, 18, 19, 21, 24, 31, 35**) failed to present the complete and entire development. Since COOPER's work on *Lilium henryi*, numerous reports indicate that the *Fritillaria*-type is not confined to the Liliaceae (**22, 28**).

In *Tulipa*, BAMBACIONI and GIOMBINI (**3**) and BAMBACIONI-MEZZETTI (**4**) recognized certain early phases, but they were unable to follow the formation of the mature megagametophyte. BAMBACIONI-MEZZETTI (**4**) did state that in *T. gesneriana* the female gametophyte degenerated before it was mature and a type of megagametophyte originated by proliferation of the cells arising from the inner integument or the nucellus. NEWTON (**33**) stated that in the megagametophyte of the Eriostemones group of *Tulipa* no fusion of megaspore nuclei took place at the chalazal end.

ROMANOV (**36**) has recently described two new forms in *Tulipa*, both of which differ from those described by NEWTON. Both forms are typical of their respective species and in both the development was analogous to the *Fritillaria*-type up to the third division. In neither species did the triple fusion of the three megaspore nuclei take place, so that in *T. rosea* a 14-nucleate gametophyte resulted and in *T. maximovicii* a 5-nucleate one; yet four divisions were required to obtain the mature megagametophyte in each species. The single megagametophyte of Inglescombe Yellow, in which eight small nuclei were found, probably arose by failure of the three megaspore nuclei to fuse. Instead, all four nuclei underwent another division to give eight approximately haploid nuclei, a total of only three divisions. Had each of these undergone a fourth division a 16-nucleate gametophyte would have resulted, which would compare with the description given for *T. rosea* above. BAMBACIONI-MEZZETTI (**5**) reported one of the 8-nucleate type but did not consider its occurrence at all common.

Inglescombe Yellow closely follows the *Fritillaria*-type of development, and four divisions are necessary to form the mature gametophyte. However, although eight nuclei may be developed, the typical megagametophyte at the time of fertilization consists of only two. Degeneration of the two synergids and the three

antipodals, leaving the polar apparatus and the egg nucleus, is the common method of development.

The first division in the megagametophyte of Inglescombe Yellow is similar to that in the pollen mother cells with respect to the chromosome associations. Trivalents, bivalents, and univalents were found, but the number of trivalents seemed to be less than those reported for the male gametophyte by WOODS (**48**). It is possible that if a larger number of complete counts were available the number of trivalents would have been greater.

In subsequent divisions in the material observed, the occurrence of lagging chromosomes and of micronuclei was negligible. It seems, therefore, that the explanation for the predominance of $3n \times 2n$ hybrids with high chromosome numbers (**6**) may be in part related to the comparative regularity of meiotic and subsequent divisions. This is in sharp contrast to the work of SATINA and BLAKESLEE (**39**) on *Datura*, where there was frequent loss of chromosomes by lagging from the female gametophyte. On the other hand, the results of their progeny studies were correspondingly different. UPCOTT and PHILP (**46**), from the chromosome numbers of $3n \times 2n$ progeny of *Tulipa*, calculated that the female gametes should contribute close to 18 chromosomes, but they made no direct observations on the megagametophyte divisions. WESTFALL (**47**) examined the formation of the megagametophyte in *Lilium tigrinum* and found that the loss of lagging chromosomes in the meiotic and following divisions produced "a general shift in the distribution toward the lower chromosome numbers." He further determined that the distribution of chromosomes at the first meiotic division gives increases toward the $1n$ and $2n$ limits of distribution.

The frequent observations of unequal division of the number of chromosomes present in any one nucleus probably explains the occurrence of $3n \times 2n$ hybrids with more or less than the mean of 30 chromosomes reported previously (**6**).

UPCOTT and PHILP (**46**) and SATINA, BLAKESLEE, and AVERY (**40**) have reported that the seeds resulting from $3n \times 2n$ crosses are poorly developed. Our observations confirm this fact. The behavior of the chromosomes in the chalazal end of the embryo sac, where both unequal division after the triple fusion and to some extent lagging chromosomes occur, probably plays an important part in the viability of the seeds. It is not to be suggested that a loss of chromosomes or unbalance brought about by unequal distribution is directly responsible for the failure of these seeds to germinate, but rather that this unbalance affects the extent to which endosperm is produced, which in turn may affect the life of the seedling during germination.

Summary

1. The megagametophyte of Inglescombe Yellow, a triploid tulip, is of the *Fritillaria*-type.

2. During the meiotic prophase of the first division trivalents, bivalents, and univalents were observed.

3. The occurrence of lagging chromosomes and micronuclei was infrequent.

4. There was unequal separation of the chromosomes in both meiotic and subsequent divisions.

5. The relation of these observations to triploid progeny is discussed.

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LITERATURE CITED

1. BAMBACIONI, V., Come avviene in *Fritillaria persica* L. lo sviluppo del gametofito femminile e l'aumento dei cromosomi nella regione calazale. Rend. Acc. Lincei Roma, Cl. Fis. Mat. Nat., Ser. 6: 544–546. 1928.
2. ———, Richerche sulla ecologia e sulla embriologia di *Fritillaria persica* L. Ann. di Bot. 18: 7–37. 1928.
3. BAMBACIONI, V., and GIOMBINI, ADA, Sullo svilluppo del gametofito femminile in *Tulipa gesneriana* L. Ann. di Bot. 18: 373–386. 1930.
4. BAMBACIONI-MEZZETTI, VALERIA, Sullo sviluppo dell'embrione in *Tulipa gesneriana* L. Ann. di Bot. 19: 145. 1932.
5. ———, Nuovo richerche sull'embriologia delle Gigliacee. Ann. di Bot. 19: 365–382. 1932.
6. BAMFORD, R., REYNARD, G. B., and BELLOWS, J. M., Jr., Chromosome number in some tulip hybrids. BOT. GAZ. 101: 482–490. 1939.
7. BELLING, J., The distribution of chromosomes in the pollen grains of a triploid hyacinth. Amer. Nat. 58: 440–446. 1924.
8. BELLING, J., and BLAKESLEE, A. F., The assortment of chromosomes in triploid Daturas. Amer. Nat. 56: 339–346. 1922.
9. CAPINPIN, J. M., Studies on the genetics and cytology of triploid Oenotheras. Cytologia 4: 355–426. 1933.
10. CARANO, E., Sul particolare sviluppo del gametofito ♀ di *Euphorbia dulcis* L. Rend. Acc. Lincei Roma, Cl. Fis. Mat. Nat., Ser. 6. 1: 733–735. 1925.
11. ———, Ulteriori osservazioni su *Euphorbia dulcis* L. in rapporto col suo comportamento apomittico. Ann. di Bot. 17: 50–79. 1926.
12. CATTORINI, P., Interno all'esistenza delle sfere direttrici o centrosfere nelle cellule del sacco embrionale della *Tulipa*. Atti Ist. Bot. Univ. Pavia, Ser. II. 13: 299–307. 1914.
13. CHANDLER, CLYDE, PORTERFIELD, W. M., and STOUT, A. B., Microsporogenesis in diploid and triploid types in *Lilium tigrinum* with special references to abortions. Cytologia, Fujii Jubilee Vol. pp. 756–784. 1937.
14. COOPER, D. C., Development of the embryo-sac of *Lilium henryi*. Proc. Nat. Acad. Sci. 20: 163–166. 1934.
15. ———, Macrosporogenesis and development of the embryo sac of *Lilium henryi*. BOT. GAZ. 97: 346–355. 1935.
16. DARLINGTON, D. C., Recent advances in cytology. 2d ed. Philadelphia. 1937.
17. DERMEN, H., Fertilization in the Baldwin apple, a triploid variety. Jour Arnold Arb. 17: 106–108. 1936.

18. ERNST, A., Beitrage zur Kenntniss der Entwickelung des Embryosackes und des Embryo (Polyembrionie) von *Tulipa gesneriana* L. Flora **88**:37–77. 1901.
19. GUIGNARD, L., L'appareil sexuel et la double fecondation dans les Tulipes. Ann. Sci. Nat. Bot. VII. **11**:365–387. 1900.
20. HALL, A. D. H., Polyploidy in *Tulipa*. Jour. Linn. Soc. Bot. **1**:481–489. 1937.
21. HEIMANS, J., Chromosomen und Befruchtung bei *Lilium martagon*. Recuéil Trav. Bot. Néerland. **25**:141. 1928.
22. JOSHI, A. C., Systematic distribution of the *Fritillaria*-type of embryo-sac and the mono- or polyphyletic origin of angiosperms. Chronica Botanica **4**:507–508. 1938.
23. LAMMERTS, W. E., Interspecific hybridization in *Nicotiana*. IX. Further studies of the cytology of the backcross progenies of the *paniculata-rustica* hybrid. Genetics **14**:286–304. 1929.
24. LANGLET, OLOF, Über die Entwicklung des Eiapparates in Embryosack der Angiospermen. Svensk Bot. Tidskr. **21**:478–485. 1927.
25. LESLEY, J. W., A cytological and genetical study of triploid tomatoes. Genetics **13**:1–43. 1928.
26. LEVAN, A., Different results in reciprocal crosses between diploid and triploid *Allium schoenoprasum* L. Nature **138**:508. 1936.
27. LONGLEY, A. E., Chromosomes in hybrids between *Euchlaena perennis* and *Zea mays*. Jour. Agr. Res. **48**:789–806. 1934.
28. MAHESHWARI, P., A critical review of the type of embryo-sacs in angiosperms. New Phytol. **36**:359–417. 1937.
29. MCCLINTOCK, B., A cytological and genetical study of triploid maize. Genetics **14**:180–222. 1929.
30. MORINAGA, T., and FUKUSHIMA, E., Cytogenetical studies in *Oryza sativa* L. II. Spontaneous autotriploid mutants in *Oryza sativa* L. Jap. Jour. Bot. **7**:207–225. 1935.
31. NAVASHIN, S., Neuen Beobachtungen über Befruchtung bei *Fritillaria tenella* and *Lilium martagon*. Bot. Centralbl. **77**:62. 1899.
32. NEBEL, B. R., Chromosome numbers in aneuploid apple seedlings. New York Agr. Exp. Sta. Tech. Bull. 209. 1933.
33. NEWTON, W. C. F., Chromosome studies in *Tulipa* and some related genera. Jour. Linn. Soc. Bot. **47**:339–354. 1927.
34. NEWTON, W. C. F., and DARLINGTON, C. D., Meiosis in polyploids. I. Triploid and pentaploid tulips. II. Aneuploid hyacinths. Jour. Genetics **21**:1–50. 1929.
35. NOTHNAGEL, MILDRED, Fecundation and formation of the primary endosperm nucleus in certain Liliaceae. BOT. GAZ. **66**:143–161. 1918.
36. ROMANOV, I. D., Two new forms of embryo-sac in the genus *Tulipa*. Compt. Rend. (Doklady) Acad. Sci. U.S.S.R. **22**:139–141. 1939.
37. SANTOS, K., Macrosporogenesis of *Lilium philippinense* Baker. Cytologia, Fujii Jubilee Vol. pp. 822–855. 1937.
38. SATINA, SOPHIA, and BLAKESLEE, A. F., Chromosome behavior in triploids of *Datura stramonium*. I. The male gametophyte. Amer. Jour. Bot. **24**:518–527. 1937.
39. SATINA, SOPHIA, and BLAKESLEE, A. F., Chromosome behavior in triploid *Datura*. II. The female gematophyte. Amer. Jour. Bot. **24**:621–627. 1937.
40. SATINA, SOPHIA, BLAKESLEE, A. F., and AVERY, A. G., Chromosome behavior in triploid *Datura*. III. The seed. Amer. Jour. Bot. **25**:595–602. 1938.

41. SCHNARF, K., Contemporary understanding of embryo-sac development among angiosperms. Bot. Rev. **2**:586–600. 1937.
42. SMITH, F. H., Reduction division in triploid *impatiens*. Amer. Jour. Bot. **25**:651–654. 1938.
43. TREUB, M., and MELLINK, J. F. A., Notice sur le développement du sac embryonnaire dans quelques angiospermes. Arch. Néerland. Sci. Exact. Nat. **15**:452–457. 1880.
44. UPCOTT, M., The genetic structure of *Tulipa*. II. Structural hybridity. Jour. Genetics **34**: 339–398. 1937.
45. UPCOTT, M., and LA COUR, L., The genetic structure of *Tulipa*. I. A chromosome survey. Jour. Genetics **33**:237–254. 1936.
46. UPCOTT, M., and PHILP, J., The genetic structure of *Tulipa*. IV. Balance, selection, and fertility. Jour. Genetics **38**:91–123. 1939.
47. WESTFALL, J. J., Cytological studies of *Lilium tigrinum*. BOT. GAZ. **101**:550–581. 1940.
48. WOODS, M. W., Meiotic studies in triploid *Tulipa* with special reference to bridging and fragmentation. BOT. GAZ. **99**:103–115. 1937.
49. WOODS, M. W., and BAMFORD, R., Chromosome morphology and number in *Tulipa*. Amer. Jour. Bot. **24**:175–184. 1937.

EFFECT OF CONCENTRATION OF OXYGEN AND PRESSURE OF WATER UPON GROWTH OF RHIZOMES OF SEMI-SUBMERGED WATER PLANTS[1]

HARLOW E. LAING

(WITH SIX FIGURES)

During the course of a study of the respiration of the rhizomes of *Nuphar advenum* and other semi-submerged plants, it was observed that these organs were able to endure low oxygen tension and even complete absence of oxygen for a considerable period of time (**7**). It was also noticed that the presence or absence of oxygen affected their rate of growth. It was decided, therefore, to study the growth attained by the shoots under different conditions of aeration.

Materials and methods

The plants used were *Nuphar advenum* Ait., *Typha latifolia* L., *Peltandra virginica* (L.) Kunth, *Pontederia cordata* L., *Acorus calamus* L., *Scirpus validus* Vahl, and *Sparganium eurycarpum* Engelm. Observations were also made on the land plants *Iris germanica* and *Ipomoea batatas*, for the sake of comparison.

The material was prepared so as to leave the buds or bases of the shoots exposed as much as possible without injury. If the petioles of a few of the large inner leaves of *N. advenum* were cut so as to leave about 3 cm. of the petioles surrounding the embryonic leaves, it was possible to control their further growth by adjusting the oxygen content of the medium. The mature rhizomes of *Typha latifolia* and *Sparganium eurycarpum* were prepared by carefully removing the terminal shoot and all the leaves cleanly to the rhizome, thus exposing the axillary buds but without injuring them. The petioles of *Peltandra virginica* and *Pontederia cordata* were cut squarely off. The fully developed membranous sheath, which remained stationary, made it possible to estimate the amount of new growth.

Except when otherwise stated, the rhizomes were put into suitable containers such as large test tubes or large-mouthed museum jars having rubber stoppers large enough to fit the tops and each fitted with two glass delivery tubes, and placed in a constant temperature bath kept at 25° C., which is about the temperature of the pond water of the native habitat during many of the warm days of summer. Moist gaseous mixtures containing various concentrations of nitrogen and oxygen from which the CO_2 had been removed were passed through the con-

[1] Paper from the department of botany of the University of Michigan, no. 721.

tainers steadily throughout each experiment, except when otherwise stated. The gases were mixed by means of a series of capillary tubes attached to manometers and governed by adjustable spill-bottles as previously described (7). The gases were drawn through the containers by a very slight negative pressure provided by an aspirator, and the pressure was kept constant by a mercury pressure regulator.

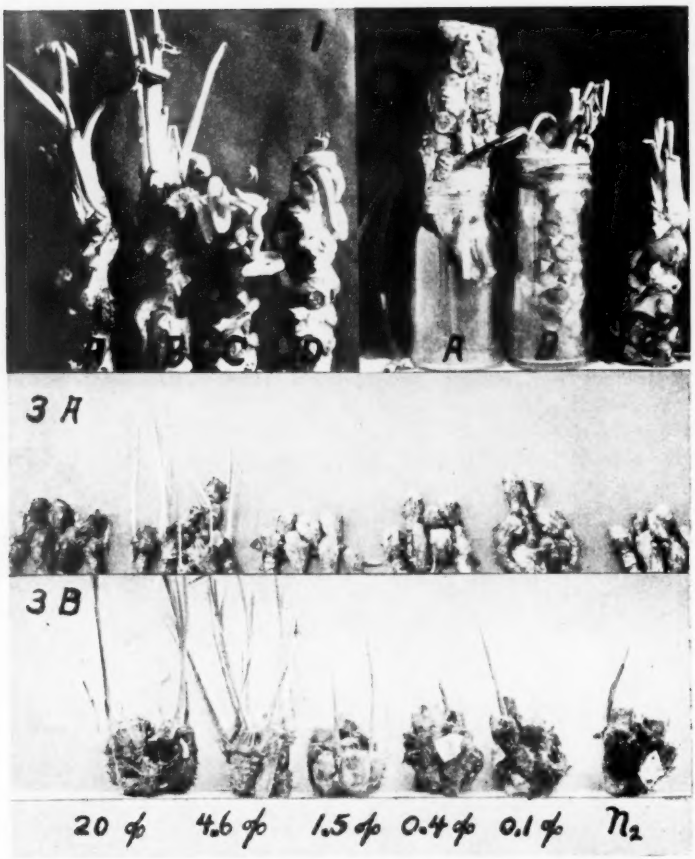
Experimental results

Effect of oxygen content of medium on growth of rhizomes

Two large rhizomes of *Nuphar advenum* were prepared as previously described, and each was submerged in water in a separate jar at room temperature. Air was bubbled through one jar and purified nitrogen through the other for 29 days. The results (fig. 1*A*, *B*) show that growth was approximately equal in both rhizomes.

To determine the effect of various concentrations of oxygen upon the growth of *N. advenum*, a number of rhizomes were prepared and subjected to concentrations of 20, 10, 4.6, 3, 1.5, 1, 0.4, and 0.1 per cent of oxygen by volume, and purified nitrogen, separately. Figure 4*A* shows that oxygen inhibits growth unless it is present only in very small quantities. The maximum rate of growth occurred in the rhizomes receiving from 1 to 0 per cent of oxygen. When the oxygen concentration exceeded 1.5 per cent, growth became very slow and ceased entirely at concentrations above 3 per cent. Leaves of *N. advenum* that had grown rapidly on rhizomes in nitrogen ceased elongating when the rhizomes were placed in air and became more stocky, the blades broadening slightly and the color changing from the previous pale etiolated condition to a glossy dark green. It was concluded that although anaerobic conditions are conducive to leaf growth, nevertheless when the leaves have once begun to unfold they are thereafter benefited by at least a small amount of oxygen. Apparently growth of *N. advenum* is not dependent upon an aqueous medium, but rather upon the fact that the aqueous medium limits the supply of oxygen.

Similar experiments were performed with the rhizomes of other species. *Peltandra virginica* and *Pontederia cordata* grew well in aerated water and in water through which nitrogen was passed, but *Typha latifolia* did not grow under either condition. When subjected to moist gaseous mixtures, *T. latifolia* grew best in an atmosphere containing 4.6 per cent of oxygen (fig. 3*A*), next best in 10 per cent, and third best in 3 per cent; no shoot growth occurred in concentrations of less than 3 per cent of oxygen and very little in 20 per cent of oxygen, that is, air. Although growth in this plant was inhibited in nitrogen and the lower concentrations of oxygen, nevertheless the buds themselves were able to endure a 13-day period of exposure to anaerobic conditions, as was shown by the following experi-



FIGS. 1–3.—Fig. 1, growth of rhizomes of *Nuphar advenum:* *A*, in aerated water; *B*, in water through which nitrogen was bubbled; *C*, under pressure of 2 feet of aerated water; *D*, under pressure of 4 feet of same. Fig. 2, growth of rhizomes of *N. advenum:* *A*, with apex in air and remainder in nitrogen; *B*, with apex in nitrogen and remainder in air; *C*, control, entirely surrounded by nitrogen. Growth occurs when apex is in nitrogen and not when it is in air, regardless of which gas surrounds remainder of rhizome. Fig. 3, growth of rhizomes of *Typha latifolia:* *A*, after 13 days in air, in various concentrations of oxygen, and in nitrogen; *B*, same rhizomes 21 days later, after having been exposed to slow stream of moist air in bell jar for 21 days.

ment. After being photographed, the rhizomes shown in figure 3*A* were carefully tied together in little bundles, each group by itself. These bundles were put into a bell jar through which moist air was passed very slowly for 21 days, at the end of which time they were photographed again while still tied together (fig. 3*B*). The subsequent growth shows that many of the buds had survived the previous lack of oxygen.

In contrast to *Typha latifolia*, both *Peltandra virginica* and *Pontederia cordata* grew well in nitrogen and the lower concentrations of oxygen (fig. 4*B*, *C*), but *P. virginica* did not grow perceptibly in concentrations of oxygen as high as 10 per cent; and although *P. cordata* grew well in 10 per cent of oxygen, it too was somewhat retarded in air.

Rhizomes of *Acorus calamus*, *Sparganium eurycarpum*, and *Scirpus validus* were exposed for 8 days to 20, 10, 3, 1.5, and 1 per cent of oxygen and nitrogen. The results are given in table 1. The fact that the rhizomes of *A. calamus* produced in low oxygen concentrations shoots that were able to live and grow through the experiment showed that these rhizomes were able to endure a considerable period of anaerobiosis. But they did not grow rapidly unless they had more than 3 per cent and somewhat less than 20 per cent of oxygen. Best growth was at 10 per cent, but probably the optimum concentration is either a little above or a little below this percentage. In *S. eurycarpum* the shoots were not only able to endure anaerobic conditions but actually grew as well in 1.5 per cent of oxygen as in moist air.

In *Scirpus validus* growth was definitely better at 10 per cent of oxygen than at the other concentrations. Growth in the lower concentrations and in nitrogen undoubtedly began before the original air of the containers had all been displaced by the incoming gases. Although it is not known how long this growth was sustained, it was certain at the end of the experiment that all the shoots had died in the jars containing only 1 per cent of oxygen and in nitrogen, and were dying in 1.5 per cent of oxygen. In another experiment, not reported here, it was found that the buds on rhizomes of *S. validus* that had remained dormant in nitrogen continuously for 8 days without injury were able to grow when placed in moist air, and that shoots that had previously been formed in moist air were killed in nitrogen.

Throughout all these experiments, observations were made of any noticeable root growth. In both *Typha latifolia* and *Pontederia cordata* the best root growth occurred in air, the next best in 10 per cent, next in 4.6 per cent of oxygen, etc.; but no root growth occurred in concentrations of less than 1.5 per cent of oxygen. In *T. latifolia* root growth practically ceased in 1.5 per cent. Toward the end of the experiment with *P. cordata* the nitrogen and air tubes were exchanged, so

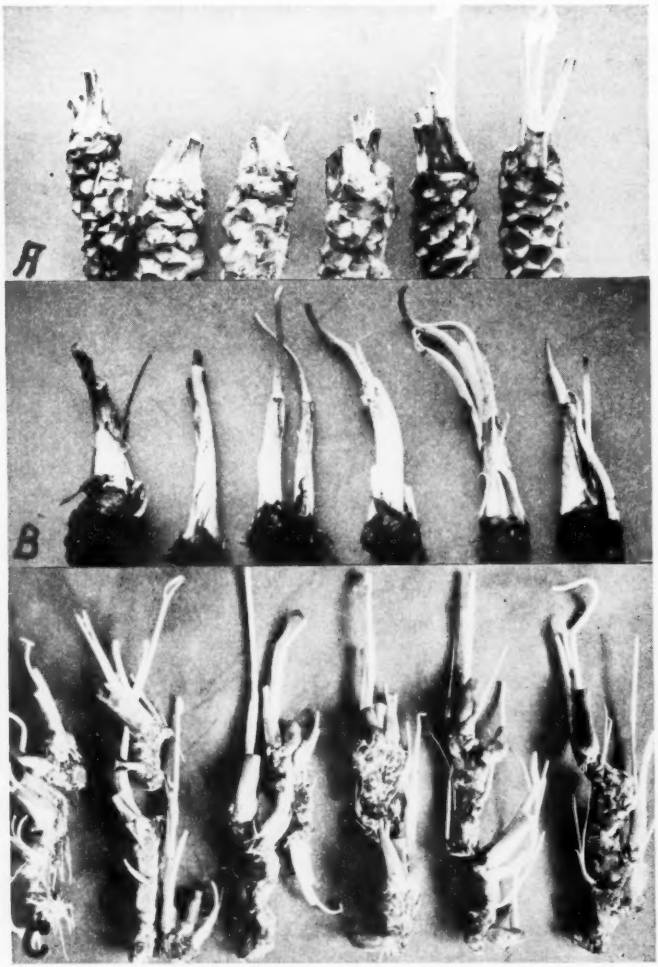


FIG. 4.—Growth of rhizomes in (reading from left to right) air, 10%, 3%, 1.5%, 1% of oxygen, and in nitrogen: *A*, *Nuphar advenum* after 11 days; *B*, *Peltandra virginica* after 8 days; *C*, *Pontederia cordata* after 11 days.

that the samples that had been in air for 8 days were continued in nitrogen for 3 days, and those that had been in nitrogen for 8 days were continued in air for 3 days. This exchange was sufficient to induce a beginning of root formation on the rhizome that had been in nitrogen previously (fig. 4*C*, N_2). On the other hand, the roots previously formed in air were injured by the nitrogen, and many of them were dying at the close of the experiment. This experiment shows that at least some of the root primordia of *P. cordata* are not injured by nitrogen, but that they do not begin to grow until there is a sufficient supply of oxygen. After growth has progressed to the extent that the roots have extended into the medium, the

TABLE 1

MAXIMUM LENGTH OF GROWTH, IN MILLIMETERS, OF SHOOTS AND ROOTS OF ACORUS CALAMUS, SPARGANIUM EURYCARPUM, AND SCIRPUS VALIDUS DURING 8 DAYS IN VARIOUS CONCENTRATIONS OF OXYGEN

SPECIES	20% O_2	10% O_2	3% O_2	1.5% O_2	1% O_2	N_2
A. calamus	65	80	45	30	25	10
S. eurycarpum	25	7	15	25	8	5
S. validus	70	120	45	30 (dying)	40 (dead)	90 (dead)

roots thereafter are very sensitive to a lack of oxygen, or at least are harmed by nitrogen from which oxygen and CO_2 have been removed.

Included with the preceding experiments were some rhizomes of *Iris germanica* and some tuberous roots of *Ipomoea batatas*. The leaves of *Iris* were cut off uniformly, leaving about 3 cm. at the base. Some young shoots 1–2 cm. in length having small expanding leaves were present on *Ipomoea* at the beginning of the experiment. The results showed that the leaves of *Iris* cannot endure the conditions of oxygenation below 3 per cent. The only growth that occurred was in air and in 10 per cent of oxygen. The leaves grew nearly as well in 10 as in 20 per cent of oxygen, but the roots grew best in 20 per cent. The leaves that had already grown on *Ipomoea* previous to the experiment were able to endure anaerobiosis to some extent but were injured at concentrations below 1.5 per cent. Root growth was good in air and in 10 per cent of oxygen.

Experiments were performed on various other species of semi-submerged water plants and also on species of land plants. The results were not different from those already obtained.

In summarizing this group of experiments it may be said that the growth of semi-submerged plants is hindered by fully aerobic conditions, but that some oxygen is favorable to the growth of certain species, the optimum being different for different species. Also the optimum range of oxygen concentration is comparatively broad for some species, such as *Pontederia cordata*, but is relatively narrow

for others, such as *Typha latifolia*, *Peltandra virginica*, and *Nuphar advenum*. One striking result is the fact that the initiation of growth in some species required no oxygen whatever, and that in *N. advenum* oxygen was detrimental to growth when present in amounts exceeding 1 per cent.

Effect of oxygen content of internal atmosphere on growth of Nuphar advenum

To determine whether or not the oxygen content of the internal atmosphere of the rhizome of *N. advenum* exerts an effect on the growth of the leaves, the following two experiments were performed.

In the first experiment analyses were made of the internal atmospheres of a number of rhizomes under different conditions. The gases were extracted at room temperature by means of an apparatus previously described (9) and analyzed in a Henderson-Haldane apparatus. The internal atmosphere of a rhizome kept in a stream of nitrogen for 10 days contained 2 per cent of CO_2 and no oxygen; that of a rhizome kept in CO_2 for 11 days contained 75 per cent CO_2 and no oxygen; that of another rhizome kept in stale water saturated with CO_2 under a slight excess pressure contained 88 per cent of CO_2 and no oxygen; while that of a rhizome lying on the laboratory table under a moist cloth contained 19.6 per cent of oxygen and only 1.2 per cent of CO_2. The air of the laboratory contained 20 per cent of oxygen. These results indicate that the composition of the internal atmosphere of a detached leafless rhizome of this plant tends to resemble that of the gaseous medium, allowing for certain slight differences due undoubtedly to respiration or fermentation.

In the second experiment two uniform rhizomes were placed in fruit jars in such a manner that only the leaves of the bud of one of the rhizomes extended above the jar (fig. 2*B*), while only the leaves of the bud of the other rhizome protruded into the other jar and the remainder of the rhizome extended above it (fig. 2*A*). The jars were made air-tight by sealing the rhizomes in with plasteline. Tubes were inserted so that moist air could be passed through each jar. The jars were then placed in a large bell jar, and the tubes were connected through a large rubber stopper so that air could be circulated through the small fruit jars, while nitrogen was being circulated through the remainder of the bell jar. An extra rhizome was placed in the bell jar to serve as a control. The results of this experiment are shown in figure 2. The terminal bud that extended into the nitrogen-filled bell jar grew, regardless of the fact that the remainder of the rhizome undoubtedly contained much oxygen since it was surrounded by an atmosphere containing 20 per cent inside the small jar (fig. 2*B*). The control in the atmosphere of nitrogen grew also (fig. 2*C*). But the rhizome, the bud of which protruded into the air-filled fruit jar, did not grow although the remainder of its bulk un-

doubtedly contained little or no oxygen since it extended into the nitrogen-filled bell jar (fig. 3*A*).

These two experiments show that the anaerobic conditions that induce growth must be applied to the exterior of the bud, and that growth of the shoot is not noticeably affected by the oxygen concentration of the internal atmosphere of the rhizome.

Effect of pressure on growth of rhizomes of Nuphar advenum

Nuphar advenum is rarely, if ever, found growing in water exceeding 6–7 feet in depth. Experiments were conducted, therefore, to determine whether pressure is a limiting factor on growth.

In the first experiment a large vigorous rhizome that had grown well in 0.1 per cent of oxygen and a recently dug, short vigorous rhizome that had been kept under moist towels for 6 days—so that aerobic dormancy was fully established—were both put into a jar and connected so that they received nitrogen gas under a reduced pressure equal to 30 inches of water less than atmospheric pressure. Five days later another short, freshly dug rhizome was put into the jar with the other two.

On the eighteenth day of the experiment no additional growth had occurred on the large rhizome that had grown previously in 0.1 per cent of oxygen, and the leaves previously produced were dying. They were still pallid, showing that no air had entered the jar. The short rhizome that was put in at the beginning of the experiment had barely begun to grow. The short rhizome that had been put in on the fifth day had not grown. It was therefore decided to increase the pressure of nitrogen to an amount equal to 24 inches of water more than atmospheric pressure. This was accomplished by the device shown in figure 5. The nitrogen gas, after passing through the respiration jar, was compelled to displace 24 inches of water before escaping in bubbles.

Within 12 days the two short rhizomes produced a growth of 30 mm. and 50 mm., respectively, showing that the previously reduced pressure of nitrogen, while hindering growth, had not destroyed the tissues. The larger rhizome failed to recover.

The next experiment employed a variety of pressures. Two rhizomes were put into each of five jars, and these were arranged so that the amount of water being displaced by the escaping nitrogen was 2, 4, 6, 8, and 10 feet respectively. Growth occurred in all the rhizomes; the average amount in the lower pressures exceeded that in the higher.

To determine how long these pressures of nitrogen could be endured, the rhizomes were returned to the jars. This time, however, one or both from each jar

were exchanged for a rhizome from another jar or for a fresh one in order to note any differences in growth. Growth occurred under all pressures, even as high as the equivalent of 10 feet of water more than atmospheric pressure, but previously formed leaves did not endure such high pressure very long. The most suitable pressures were 2, 4, and 6 feet.

This experiment was repeated in a medium of aerated water instead of nitrogen gas, by means of the device shown in figure 6. The continuous column of

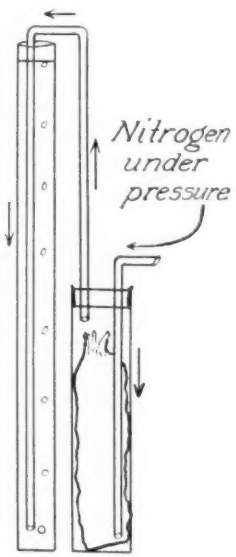


FIG. 5

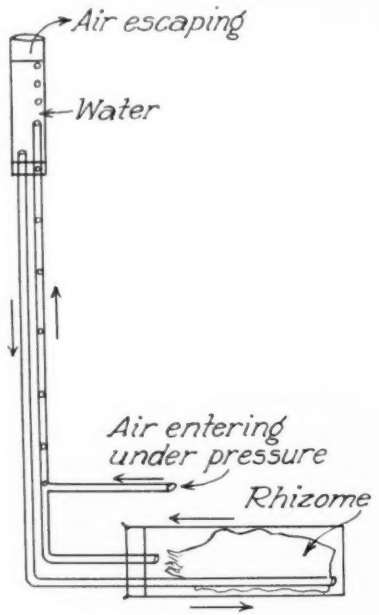


FIG. 6

FIGS. 5, 6.—Fig. 5 (left), device used to produce excess pressure of nitrogen equal to given number of feet of water pressure. Fig. 6 (right), device used to produce effective controlled pressure of given number of feet of aerated water.

water, circulating through the two tubes opening into an elevated reservoir above and into the respiratory jar lying horizontally below, was kept in motion and aerated by air under pressure which, rising in bubbles, forced the column of water to move as indicated by the arrows. Dye was introduced to make certain that the water was actually circulating. The desired pressure was obtained by adjusting the distance of elevation between the reservoir and the respiratory jar. The pressures used were 2, 4, 6, 8, and $9\frac{1}{2}$ feet of water. Best growth occurred in 2 and 4 feet, but growth was very satisfactory at the higher pressures.

The results of another experiment conducted similarly are shown in figure 1*C*, *D*. The rhizomes *C* and *D* made excellent growth of leaves in 2 and 4 feet of water, respectively, during 18 days. The contortion of the petioles is due to their cramped position in the closed horizontal jars. There was no noticeable difference in the average daily rate of growth of these two rhizomes and the two previously described that were not subjected to pressure (fig. 1*A*, *B*).

These experiments show that pressure up to $9\frac{1}{2}$ feet of water does not prevent growth, but that growth is better under pressure not exceeding 4–6 feet.

Discussion

From these experiments it may be concluded that the adaptation of the different species of semi-submerged water plants to various depths of water is at least partly due to the different oxygen requirements for maximum growth of the young shoots. Although *Typha latifolia*, *Sparganium eurycarpum*, *Scirpus validus*, and *Acorus calamus* were able to respire anaerobically and to endure anaerobic conditions in the dormant state, nevertheless they required appreciable amounts of oxygen to sustain growth and respiration in the active state. This is associated with the observation that these plants normally flourish in comparatively shallow water and are also to be found growing on well-aerated muddy shores that are inundated only part of the season. In fact, the manner of growth of *T. latifolia*, *S. eurycarpum*, and *A. calamus* is such that usually some of the shoots are in contact with air the year round. In winter, when the old leaves have died, young green shoots may be found pressed in between the old leaves and extending above the ice. In the latitude of Chicago and Detroit, these shoots stay green all winter and the more vigorous ones give rise to fruiting culms the following summer. It is undoubtedly this need of oxygen that prevents their successful invasion of deep water. It must not be forgotten, however, that growth was found to be retarded by too much oxygen as well as by too little. This is associated with the observation that these plants, while able to grow for some time on well-aerated muddy shores, nevertheless do not succeed so well there as in shallow marshy water, and, unless inundation occurs occasionally, the colonies eventually disappear.

The retardation of growth in air of the young shoots of *Nuphar advenum*, *Peltandra virginica*, and *Pontederia cordata* was even more pronounced than in the previously mentioned species. This is associated with the observation that these plants normally grow best where the water is at least 12 inches deep, and that growth becomes promptly retarded and soon ceases altogether on well-aerated muddy shores left by the subsidence of the water level. *N. advenum* seems to be affected more quickly than the others. This agrees with the fact that it is the most sensitive to oxygen. A somewhat similar condition was found in *Limnanthemum nymphaeoides* by FUNKE (5), who reported that the young leaves grew best

when the oxygen content of the water was restricted and poorest when the water was well aerated. This injury to growth by too much oxygen is not confined merely to water plants. In certain unpublished studies, the writer found that buds of *Aesculus hippocastanum* and various other trees and shrubs from which the scales had been carefully removed grew readily in an atmosphere containing 10 per cent of oxygen but were greatly retarded in air. KIDD (**6**) and THORNTON (**11**) found that the thin-skinned freshly harvested tubers of potatoes sprouted readily in atmospheres containing 2–10 per cent of oxygen, but not in air. WENT and THIMANN (**13**) stated that growth hormones are easily oxidized and rendered functionless, but whether or not that accounts for the retarding effect of oxygen on growth is uncertain.

Although the young leaves of *Nuphar advenum* grew as rapidly in nitrogen as in 1 per cent of oxygen, those growing in nitrogen remained etiolated until oxygen was introduced, but growth was retarded when the oxygen concentration exceeded 1 per cent. As is pointed out elsewhere (**8**), mature aerial leaves of *N. advenum* succumbed to the lethal effects of anaerobic conditions about as soon as did those of *Pelargonium zonale*. The exact nature of the change of adaptation of these leaves from anaerobic to aerobic conditions is unknown.

A difference in respect to the resistance of leaves of different species to anaerobic conditions was also observed. Similar observations have been made by DEHERAIN and MOISSAN (**3**) and their practical importance pointed out by SCURTI and ZAVANAJU (**10**).

Although root growth was not studied primarily, it was observed that the newly formed roots of *Pontederia cordata* were killed when subjected to an atmosphere of pure nitrogen, and that, regardless of the species, the best root growth invariably occurred in concentrations of 10 or 20 per cent of oxygen. Similar observations have been made by other investigators (**2, 12, 4, 1**). It should be pointed out that although roots grow well in moist air, that in itself is no reason for supposing that such high concentrations of oxygen are essential for root growth. Just why the young roots of such a species as *Typha latifolia* should thrive in moist air while the young shoots are markedly retarded is unknown.

Another significant point brought out in these experiments is the fact that the anaerobic conditions that induce growth in *Nuphar advenum* must be applied to the exterior of the bud, and that the growth of the shoot is not noticeably affected by the oxygen concentration of the internal atmosphere of the rhizome. This fact is of distinct physiological and ecological interest because, as has been shown by other experiments (**9**), during the growing season the internal atmosphere of the apex of the rhizome—which at night may contain only a very small amount of oxygen—in the daytime may contain as much as 10 per cent, obtained chiefly as a by-product of photosynthesis. This internal oxygen is respired within the rhi-

zome, and little if any can enter the very young leaf because of the numerous dense layers of undifferentiated tissue that compose the abscission zone at this stage. As the petiole grows the air cavities become enlarged and more extensive, until finally air passages are opened up through the abscission zone and gases pass freely between the rhizome and the petiole.

Besides the oxygen tension, it was also found that the pressure exerted upon the rhizomes slightly influenced the development of new shoots. A reduced pressure equal to 30 inches of water less than atmospheric pressure inhibited growth of the young leaves of *N. advenum* in an atmosphere of moist nitrogen. The young leaves, however, grew in the atmosphere of moist nitrogen under positive pressure equal to as much as 10 feet of water. The average growth, however, was better at 2, 4, and 6 feet than at 8 and 10 feet. Similarly in aerated and non-aerated stale water, pressure equal to $9\frac{1}{2}$ feet did not prevent growth, but growth was best at 2, 4, and 6 feet. It is concluded that water pressure, in itself, is not the factor that prevents the migration of the colonies into deeper water.

Summary

1. Semi-submerged water plants inhabit their particular habitats very largely on the basis of adaptation in respect to their oxygen requirements for respiration and growth. The oxygen requirements for growth are different for different species.

2. The percentage of oxygen found to be most conducive to the growth of young shoots in certain of the species studied was, respectively, *Nuphar advenum* 0 to 1, *Peltandra virginica* 0 to 1.5, *Pontederia cordata* 0 to 10, *Typha latifolia* 4.6, *Acorus calamus* 10, *Scirpus validus* 10. Buds of all these species were able to endure prolonged exposure to pure nitrogen, but no growth occurred in *A. calamus*, *S. validus*, and *T. latifolia* in nitrogen. Shoots of *S. validus* previously formed were killed in nitrogen.

3. The excellent growth of *N. advenum* in nitrogen was not affected by either the presence or absence of oxygen in the internal atmosphere of the rhizome so long as the apex was surrounded by nitrogen. When the apex was surrounded by air, no growth occurred even though the rest of the rhizome was surrounded by nitrogen.

4. Even though the shoots of most of the water plants studied grew best in a medium having a low oxygen content and rarely in one having a high content, nevertheless the roots invariably grew best when the oxygen content was high and never when it was very low. Roots previously formed in air were killed in nitrogen.

5. Pressure of water as high as $9\frac{1}{2}$ feet did not prevent growth in *Nuphar advenum*, but growth was usually better under pressures not exceeding 4–6 feet.

A pressure of nitrogen gas equivalent to 10 feet of water did not prevent growth, but the young leaves were unable to endure this high pressure for as long as they could endure pressures equivalent to 2 and 4 feet. Growth did not occur in a reduced pressure of nitrogen gas equal to 30 inches of water less than atmospheric pressure.

This investigation was conducted under the direction of Dr. F. G. GUSTAFSON, to whom the writer is indebted for various suggestions and encouragement. He also wishes to thank Drs. C. D. LA RUE and C. C. MELOCHE for helpful information. During part of the time that this research was conducted, the writer was the holder of an F. C. and Susan Eastman Newcombe fellowship in plant physiology.

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LITERATURE CITED

1. CONWAY, VERONA M., Studies in the autecology of *Cladium mariscus* R. Br. New Phytol. **36**:64–96. 1937.
2. DEAN, ELIZABETH B., Effect of soil type and aeration upon root systems of certain aquatic plants. Plant Physiol. **8**:203–222. 1933.
3. DEHERAIN, P. P., and MOISSAN, H., Researches sur l'absorption d'oxygène et l'émission d'acide carbonique par les plantes maintenues dans l'obscurité. Ann. Sci. Nat. Ser. 5. **19**: 321–357. 1874.
4. EKAMBARAM, T., Presidential address before the Indian Botanical Society (on the subject of root-atmospheres). Jour. Ind. Bot. Soc. **11**:99–102. 1932.
5. FUNKE, G. L., Observations on the growth of water plants. Biol. Jaarboek deel I–XXV, pp. 382–403. Antwerp. 1938. (Ghent Botanical Institute, Sept. 1938.)
6. KIDD, F., Laboratory experiments on the sprouting of potatoes in various gas mixtures. New Phytol. **18**:248–252. 1919.
7. LAING, H. E., Respiration of the rhizomes of *Nuphar advenum* and other water plants. Amer. Jour. Bot. **27**:574–581. 1940.
8. ———, Respiration of the leaves of *Nuphar advenum* and *Typha latifolia*. Amer. Jour. Bot. **27**:583–585. 1940.
9. ———, The composition of the internal atmosphere of *Nuphar advenum* and other water plants. Amer. Jour. Bot. **27**:861–868. 1940.
10. SCURTI, F., and ZAVANAJU, A., The behavior of flowers in various gases. Ann. sper. agrar. (Rome) **21**:25–50. Jour. Amer. Chem. Soc. Abstr. **31**:8606. 1936.
11. THORNTON, N. C., Oxygen regulates the dormancy of the potato. Abstract of paper presented before physiological section of Botanical Society of America. Richmond, Virginia, 1938.
12. WEAVER, J. E., and HIMMEL, W. J., The relation of increased water content and decreased aeration to root development in hydrophytes. Plant Physiol. **5**:69–92. 1930.
13. WENT, F. W., and THIMANN, K. V., Phytohormones. New York. 1937.

PHASIC DEVELOPMENT OF MARQUIS SPRING WHEAT AND FULHIO WINTER WHEAT

D. J. WORT

(WITH TWO FIGURES)

Introduction

The theory of phasic development of plants assumes that progress toward reproductive maturity consists of a series of phases occurring in strict sequence. The environment favorable for completion of one phase may be altogether unsuitable for the initiation and progress of the subsequent phase. No matter whether conditions are favorable for the completion of later phases, the plant cannot advance toward reproductive maturity unless the previous stage has been completed.

An analysis of the literature suggests the existence of at least four phases, which in order are: (1) prethermo-phase or phases, (2) thermo-phase, (3) scoto- or dark-phase, and (4) photo-phase. The photo-phase may consist of a flowering phase and a gametogenesis phase. Of the four, the thermo- and photo-phase have received most emphasis.

The experiments described here were designed to investigate (1) the thermo-phase of Marquis spring wheat in relation to its total or partial completion during seed ripening, and the effect of this completion on the response of the ripened seed to vernalization treatment; (2) the scoto-phase of Marquis wheat and Fulhio winter wheat, and the possibility of its completion under an 8-hour photoperiod; and (3) whether any portion of the photo-phase of these wheats could be passed as quickly under an 8-hour photoperiod as under continuous light.

Methods

The procedure for vernalization and criteria for heading and flowering are those already outlined (**16**). The vernalization formulas are indicated in each section. Controls received the same amount of water as the treated seeds and were then allowed to germinate for 24 hours at 20° C.

Five plants were grown in $4\frac{1}{2}$-inch pots filled with well-mixed loam soil. When an 8-hour photoperiod was used the plants were placed in complete darkness for 16 hours out of each 24-hour period by covering with black cloth, or trucks holding the pots were run into lightproof compartments. Sixteen-hour photoperiods (16 hours of light followed by 8 hours of darkness) and 24-hour photoperiods (con-

tinuous light) were obtained by supplementing the natural day length with light from 200-watt Mazda filament lamps suspended 24 inches above the tops of the pots.

Vernalization treatments were begun at such times so that all were concluded on the same day, February 24, 1940, for the original experiments and April 12, 1940, for the repetition of the experiments.

Thermo-phase of Marquis wheat

To obviate the possibility of natural vernalization of seed during ripening (**4, 6, 17**), Marquis spring wheat was grown in the greenhouse during the autumn of 1939. The temperature in the greenhouse did not fall below 21.1° C. (70° F.) at any time. At the soft dough stage a number of pots were placed in a coldroom, the temperature of which was 1.9 ± 0.9° C. The plants were not illuminated. Pots of wheat were removed from the cold chamber at the end of 2, 4, 6, 8, and 10 days and returned to the greenhouse to ripen.

These seeds, together with seeds of plants which had not experienced vernalization temperatures during growth, were vernalized for 0, 4, and 8 days at 3° C., the water added being 60 per cent of the air-dry weight. Seven pots of five plants each were grown for each combination of treatments, a total of 630 plants in all. These were grown in a greenhouse, the minimum temperature of which was 21.1° C., under a photoperiod of 16 hours.

Scoto-phase of Marquis spring wheat and Fulhio winter wheat

The seed of Marquis spring wheat, the ripening conditions of which were unknown, was vernalized using the formula 8:3:60 (8 days chilling at 3° C., the water added being 60 per cent of the air-dry weight of seed), and Fulhio winter wheat using the formula 36:3:40.

Forty-two pots of vernalized and forty-two pots of unvernalized Marquis wheat were placed in a darkroom, the temperature of which was 21.1° C. or above, and at the end of 4, 8, and 12 days following emergence removals of pots were made to a 16-hour and to a 24-hour photoperiod. Likewise fifty-six pots of both vernalized and unvernalized Fulhio winter wheat were placed in the darkroom; and at the end of 5, 10, 15, and 20 days following emergence, removals of pots were made to a 16-hour and to a 24-hour photoperiod. Each removal involved seven pots of vernalized and seven pots of unvernalized wheat.

Seventy-seven pots of vernalized and seventy-seven pots of unvernalized Marquis and 133 pots of vernalized and 133 pots of unvernalized Fulhio wheat were placed on the 8-hour bench. Removals to a 24-hour photoperiod were made at intervals of 5 days following emergence. This continued for 40 days (eight trans-

fers) in the case of Marquis and for 75 days (fifteen transfers) in the case of Fulhio wheat. Similar transfers were made to a 16-hour photoperiod, the intervals being 5, 10, and 15 days following emergence in the case of Marquis and 5, 10, 15, and 20 days in the case of Fulhio wheat.

In a repetition of the experiment (experiment B), Marquis wheat, vernalized and unvernalized, was sown on April 12, 1940. Transfers from darkness to continuous light were made at the end of 0, 4, 7, and 10 days following emergence. No 16-hour photoperiod was used.

Photo-phase of Marquis and Fulhio wheats

The environment to which the seeds used in this section were exposed during ripening is not known. The formula used in vernalizing the Marquis wheat was 8:3:60; for the Fulhio wheat, 36:3:40. The plants were grown on a 24-hour photoperiod. Five days after emergence, seven pots of unvernalized and seven pots of vernalized Marquis and Fulhio wheat were removed from the 24-hour to an 8-hour photoperiod. Removals were made every 5 days thereafter until 35 days had elapsed in the case of Marquis and 75 days in the case of Fulhio. In a repetition of the experiment, Marquis wheat was sown on April 12. Removals were made 4 days after emergence and every 3 days thereafter for a total of 28 days, thus involving 700 plants.

Results

Thermo-phase of Marquis spring wheat

The results of the investigation of the thermo-phase of Marquis spring wheat are given in table 1. The average number of days to head and flower are given to the nearest whole number. The seeds which were not vernalized and which were obtained from parent plants which had not experienced temperatures below 21.1° C. (and hence presumably had not been vernalized) were considered the controls. Flowering of plants grown from control seed occurred 57 days after planting.

Vernalization of the control seed for 4 and 8 days produced an acceleration in flowering of 6 and 4 days, respectively. Treatment of the plants with cold for 2, 4, 6, 8, and 10 days when the grain was ripening accelerated flowering of plants subsequently grown from the seed by 1–2 days in all cases except one. Apparently the acceleration caused by vernalization of seed from chilled plants is less than that from unchilled.

To eliminate as far as possible the role of continuous darkness and constant low temperature during chilling of the ripening seed, Marquis spring wheat was grown in the field during the summer and autumn of 1939, and early-ripening and

late-ripening plants harvested separately. The air and soil temperatures for the entire growing season were recorded by thermographs.

Flowering and heading times of the 300 plants grown in the greenhouse from the seed collected in the field are shown in table 2. The early-ripened seed, which experienced little if any chilling during ripening, responded to vernalization treat-

TABLE 1

EFFECT ON HEADING AND FLOWERING OF PLANTS OF MARQUIS SPRING WHEAT GROWN FROM SEED CHILLED DURING RIPENING AND VERNALIZED PRIOR TO PLANTING

No. of days plant chilled during seed ripening	No. of days seed vernalized prior to planting	Average no. of days to head	Average no. of days to flower	Acceleration of flowering due to chilling of plant	Acceleration of flowering due to vernalization of seed	Total acceleration of flowering	No. of plants
0	0	53	57	.....	.....	.....	35
2	0	50	55	2	.....	2	35
4	0	54	55	2	.....	2	35
6	0	56	57	0	.....	0	35
8	0	54	56	1	.....	1	35
10	0	55	56	1	.....	1	35
0	4	50	51	.....	6	6	35
2	4	50	51	2	4	6	35
4	4	52	52	2	3	5	35
6	4	52	53	0	4	4	35
8	4	54	55	1	1	2	35
10	4	49	51	1	5	6	35
0	8	51	53	.....	4	4	35
2	8	50	53	2	2	4	35
4	8	54	55	2	0	2	35
6	8	53	54	0	3	3	35
8	8	54	55	1	1	2	35
10	8	54	55	1	1	2	35
Total plants							630

ment for 4 and 8 days with accelerations of flowering of 4 and 6 days, respectively. The late-ripening grain, which had experienced air temperatures of 10° C. or below during 8 days and soil temperatures of 10° C. or below during 21 days, was less sensitive to treatment. The accelerations of flowering were 2 and 3 days, respectively.

The claim of KOSTJUCENKO and ZARUBAILO (**6**), GREGORY and PURVIS (**4**), and others that vernalization can occur during seed ripening is substantiated by these laboratory and field results. That the thermo-phase was not entirely com-

pleted during ripening, under the conditions of these experiments, was evident from the fact that a positive response to vernalization of the seed was obtained.

Scoto-phase of Marquis and Fulhio wheats

Exposure of unvernalized and vernalized Marquis spring wheat to darkness for 4–12 days after emergence from the soil and then transferring to a 24-hour photoperiod did not decrease the total time required for flowering. The results of experiment A (planted February 24), involving 1190 plants, and of experiment B

TABLE 2

HEADING AND FLOWERING DATA OF PLANTS GROWN FROM VERNALIZED AND UNVERNALIZED MARQUIS WHEAT SEED PRODUCED IN THE FIELD

When ripened in field	Days seed vernalized prior to planting	Average no. of days to head	Average no. of days to flower	Acceleration of flowering due to vernalization of seed	No. of plants
Early	0	53	54	.....	50
Early	4	48	50	4	50
Early	8	47	48	6	50
Late	0	53	53	.....	50
Late	4	49	51	2	50
Late	8	48	50	3	50
Total plants					300

(planted April 12), involving 490 plants, are given in table 3. The results of experiment A are shown graphically in figure 1. Thirty-five vernalized and thirty-five unvernalized plants were grown at each treatment. Only the data for unvernalized plants are included, as vernalization caused no constant acceleration of flowering. The heading and flowering times are the average for the first twenty plants to head and to flower.

Irrespective of the length of exposure to darkness, all plants required approximately the same number of 24-hour photoperiods to flower: forty in experiment A, thirty-eight in experiment B. The total time from emergence to flowering was increased by the length of exposure to darkness.

When an 8-hour photoperiod was substituted for darkness, the shorter exposures had little effect on the number of 24-hour photoperiods subsequently required to produce flowering of Marquis wheat. Longer exposure to an 8-hour photoperiod reduced the number of 24-hour photoperiods required by two to five (table 3).

As in the case of exposure to darkness, the total number of days from emergence to flowering was increased by the 8-hour treatment. Results similar to those involving a 24-hour photoperiod were obtained when darkness or an 8-hour photoperiod was followed by a 16-hour photoperiod (table 3).

TABLE 3

EFFECTS OF DARKNESS AND 8-HOUR PHOTOPERIOD FOLLOWING EMERGENCE ON TIME TO HEAD AND TO FLOWER BY MARQUIS SPRING WHEAT AND FULHIO WINTER WHEAT

Experiment	Days of darkness after emergence	Additional 24-hour days		Total days to flower	Additional 16-hour days		Total days to flower	No. of 8-hour days after emergence	Additional 24-hour days		Total days to flower	Additional 16-hour days		Total days to flower
		To head	To flower		To head	To flower			To head	To flower		To head	To flower	
		Marquis spring wheat												
A	0	35	40	40	52	54	54	0	35	40	40	52	54	54
	4	34	42	46	50	52	56	5	38	43	48	49	50	55
	8	37	40	48	52	54	62	10	37	41	51	46	49	59
	12	35	40	52	50	53	65	15	37	41	56			
								20	37	39	59			
								25	36	38	63			
								30	33	36	66			
								40	33	35	75			
B	0	34	37	37				0	34	37	37			
	4	37	39	43				4	34	36	40			
	7	36	38	45				7	34	35	42			
	10	37	38	48				10	34	35	45			
		Fulhio winter wheat												
	0	76	77	77	*	.....	.....							
	5	56	58	63	76	78	83							
	10	66	67	77	75	75	85							
	15	63	64	79	74	74	89							
	20	55	60	80	*	.....	.....							

* Did not head in 90 days.

The experiment using Fulhio winter wheat ran for 90 days following planting. Only vernalized plants headed and flowered. Heading and flowering data are given in table 3. The necessity for a dark-phase following the thermo-phase seems evident. Exposure to a 5-day dark period following emergence and then transfer to a 24-hour photoperiod caused an acceleration of 14 days in flowering, compared with those vernalized plants grown continuously in a 24-hour photoperiod. Ten

dark days required a supplement of sixty-seven 24-hour photoperiods to cause flowering in a total of 77 days, the same as that required by the controls exposed to 24-hour photoperiods continuously. Exposure to darkness of longer than 10 days increased the total time to flower.

FIG. 1.—Effect of darkness and of exposure to 8-hour photoperiods following emergence on number of days of continuous illumination required to cause flowering in Marquis spring wheat. Narrow lines represent number of 8-hour days or darkness; broad lines, number of days of continuous light. Total number of days from emergence to flowering obtained by addition of the two periods.

PHOTO-PHASE OF MARQUIS AND FULHIO WHEATS

The results of the two experiments on the photo-phase of Marquis wheat are given in table 4. The figures for heading and flowering are the average for the first twenty plants to head and flower, except where stated otherwise. Only data

for unvernalized plants are included, as the results of vernalization were not significant.

In experiment A (planted February 24) the adverse effects of low light intensity were shown by retardation or prevention of heading and flowering after transfer to the 8-hour photoperiod. Planting for experiment B was on April 12. The intensity of daylight was much greater, and for that reason the results are probably due to photoperiod. When the plants received fewer than twenty-two 24-hour photoperiods before being transferred to the 8-hour photoperiod, heading and

TABLE 4

HEADING AND FLOWERING DATA OF MARQUIS SPRING WHEAT RECEIVING CONTINUOUS LIGHT FOLLOWED BY 8-HOUR PHOTOPERIODS. PLANTS GROWN CONTINUOUSLY IN 24-HOUR PHOTOPERIOD REQUIRED 35 DAYS TO FLOWER IN EXPERIMENT A; 37 DAYS IN EXPERIMENT B

EXPERIMENT A			EXPERIMENT B			
NO. OF 24-HOUR DAYS AFTER EMERGENCE	ADDITIONAL 8-HOUR DAYS TO HEAD	ADDITIONAL 8-HOUR DAYS TO FLOWER	NO. OF 24-HOUR DAYS AFTER EMERGENCE	ADDITIONAL 8-HOUR DAYS TO HEAD	ADDITIONAL 8-HOUR DAYS TO FLOWER	TOTAL DAYS TO FLOWER
0	.....	.....	0	.....	.....	.....
5	.....	.....	4	.....	.....	.....
10	.....	.....	7	.....	.....	.....
15	.....	.....	10	.....	.....	.....
20	50*	.....	13	.....	.....	.....
25	43	†	16	.....	.....	.....
30	41	‡	19	§	.....	.....
			22	16	18	40
			25	12	13	38
			28	8	9	37

* Only twelve headed.
† Seven flowered.
‡ Five flowered.
§ Only one headed within 45 days.

flowering were prevented or did not occur in 45 days, the duration of experiment B. Those plants receiving twenty-eight 24-hour photoperiods following emergence flowered as quickly when transferred to the 8-hour day as those receiving 24-hour photoperiods continuously, the total flowering time being 37 days in both cases. Apparently, therefore, the continuous light requirement of Marquis spring wheat is completed in approximately 28 days; heading, flowering, and fruiting occurring in an 8-hour photoperiod thereafter as quickly as in a 24-hour photoperiod.

An experiment using 1400 plants failed to detect any substantial portion of the photo-phase of Fulhio winter wheat which can be passed as quickly in an 8-hour photoperiod as in continuous light.

Discussion

The fact that some seeds, such as *Lupinus polyphyllus*, *Vicia picta*, cabbage, tomato, turnip, swede, and beetroot (13), do not respond to vernalization and

yet the young plants produced do respond to treatment suggests that the thermo-phase is by no means the first phase in the development of the plant from the division of the zygote to the production of seed. During seed ripening it is possible that the phase or phases prior to the thermo-phase may not be completed, and therefore the thermo-phase cannot begin until the prethermo-phase is completed in the young plant. Vernalization treatment would be ineffective until the pre-thermo-phase was completed.

The length of the thermo-phase has been found to vary, not only with species but also with varieties. DOLGUSIN (**2**) has subdivided wheats into five groups in respect to the optimum temperature and the time required at those temperatures to vernalize (or complete) the thermo-phase:

GROUP	TEMPERATURE (° C.)	TIME IN DAYS
1. Early spring	8–15	5– 8
2. Late spring	3–6	10–15
3. Intermediate	2–5	20–25
4. Winter	1–4	30–35
5. Extreme winter	0–3	30–45

Apparently the higher the temperature of vernalization, the shorter its period.

Investigations of KOSTJUCENKO and ZARUBAILO (**6**), GREGORY and PURVIS (**4**), and others showed that under special conditions, for instance those prevailing in a cool northern summer and autumn, even the wheats of groups 4 and 5 can be completely vernalized during seed ripening. WORT (**17**) pointed out that those samples of a variety of spring wheat grown in regions where the ripening temperatures were relatively high responded to treatment to a greater degree than those ripened in a cooler climate, the suggestion being that the thermo-phase had been partially completed in the latter case. That partial completion of the thermo-phase is possible during ripening of the seed has been shown in the experiments described here, but under the conditions of these experiments total completion of this phase during ripening was not accomplished.

MACKOV (**12**) and EREMENKO (**3**) report that prolongation of natural daylight with supplementary illumination at night failed to accelerate flowering of wheat if the treatment were supplied during the first week or two after emergence. Subsequent to this initial period both plants exhibited acceleration of flowering by treatment with supplementary illumination. It was concluded that a stage (scoto-phase) exists at which both spring and winter wheats require high temperatures (in contrast with the thermo-phase) and darkness (in contrast with the photo-phase). The work of MCKINNEY and SANDO (**11**) could be interpreted to have established the succession thermo-phase, scoto-phase, photo-phase for winter wheats.

The experiments with Marquis wheat described by the writer failed to detect the presence of the scoto-phase or a phase satisfied by short days immediately

following the thermo-phase. In the case of vernalized Fulhio winter wheat, a definite acceleration of flowering was obtained when the young plants were grown in darkness at 21.1° C. and above for 5 days following emergence from the soil. Here the sequence is thermo-phase, scoto-phase, photo-phase. The possibility of the full or partial completion of the scoto-phase during seed ripening and later during germination must not be overlooked.

Following the scoto-phase, vernalized plants of winter wheat require light and a high temperature. Early work by MELJNIK (LYSENKO, 9) in experiments with

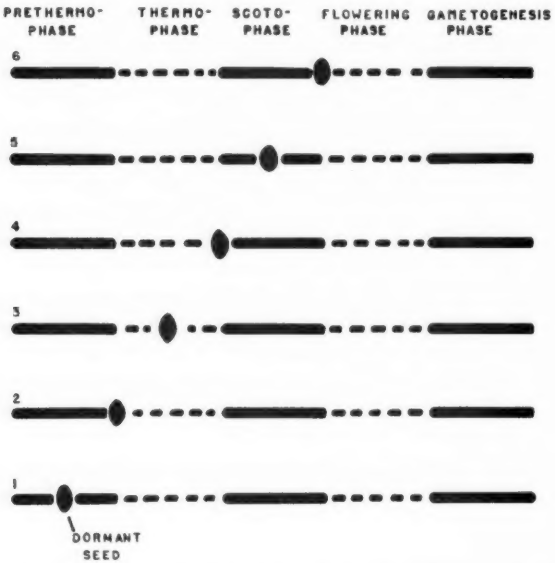

FIG. 2.—Phases of development and possible relation of dormant seed to stages in life history. Various amounts of prethermo-phase, thermophase, and scoto-phase have been completed prior to seed dormancy, as indicated by position of elipse representing dormant seed. Vernalization of seed would be most effective in 2 and less so in 3, seed of all other clasess would not respond.

winter wheat grown at high temperatures showed that vernalized plants grown first in a continuous day for not less than 20 days eared as rapidly in a 10-hour day as those kept in a continuous day throughout; that is, after 20 continuous days long or continuous light is not obligatory, and the long day is indispensable only for a definite part of the period following the thermo-phase (photo-phase). The existence of at least two parts to the photo-phase (gametogenesis phase and flowering phase) was suggested also by KIRICENKO (5) and KRAEVOI (7).

The experiments indicate that such a division of the photo-phase is possible in the case of Marquis spring wheat. The failure of the experiment using Fulhio

winter wheat to detect any portion of the photo-phase which can be passed as quickly in an 8-hour as in a 24-hour photoperiod may have been caused—at least in part—by the low light intensities prevailing during the initial stages of the experiment. Further discussion of the photoperiodic aspects of phasic development may be found in a number of recent papers (**1, 8, 10, 14, 15**).

Figure 2 graphically summarizes the various phases of development and the possible relation of the stages in the life history of the plant to the dormant seed. No attempt has been made to portray the relative lengths of the various phases.

The complete developmental history, based upon the present literature, may consist of prethermo-phase, thermo-phase, scoto-phase, and a photo-phase consisting of the flowering phase and the gametogenesis phase. A number of these phases may be completed by the time the seed has reached dormancy, depending on the species and variety of the plant and on the environment which acts on the ripening seed. In 1, only part of the prethermo-phase has been completed prior to seed dormancy. Vernalization of such seed would be ineffective, but vernalization of the plants after the prethermo-phase has been passed would be effective. The plants quoted from VASILJEV'S paper may belong in this class. In 2 the prethermo-phase has been completed by the ripening seed and the dormant seed is ready to begin the thermo-phase upon germination. Vernalization of such seed would produce the greatest effect.

Not only has the prethermo-phase been completed prior to seed dormancy in 3, 4, 5, and 6, but also varying amounts of the thermo- and scoto-phase. Those seeds which had been partially vernalized during ripening, and which responded to vernalization prior to planting by a smaller acceleration of flowering than those from plants which had not experienced chilling temperatures, doubtless belong to class 3. No response to vernalization of the seed would be expected in 4, 5, and 6, as the thermo-phase has been completed prior to dormancy.

Summary

1. By chilling the parent plants, the ripening seed of Marquis spring wheat can be at least partially vernalized.

2. No dark-phase or phase satisfied with 8-hour photoperiods immediately following the thermo-phase of Marquis wheat was detected, but exposure of Fulhio winter wheat to a 5-day dark period following emergence and then transfer to continuous light caused an acceleration of flowering compared with those vernalized plants grown in a 24-hour photoperiod.

3. Plants of Marquis spring wheat receiving twenty-eight 24-hour photoperiods following emergence flowered as quickly when transferred to an 8-hour photoperiod as did those receiving continuous light. This suggests that the photo-phase of Marquis wheat may consist of at least two parts, the first requiring long or con-

tinuous day, the second being satisfied with a short or 8-hour photoperiod. No such division of the photo-phase of Fulhio winter wheat was detected, flowering occurring most rapidly in continuous day throughout.

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LITERATURE CITED

1. ANONYMOUS, Phasic development of plants. Herb. Rev. **7**:27–32; 94–99; 181–189; 265–274. 1939.
2. DOLGUSIN, D. A., The world's collection of wheats on the background of vernalization. Seljhozgiz, Moskva. 1935.
3. EREMENKO, V. T., Intermediate period of development and anatomic-morphological characters of light state in wheat. Compt. Rend. (Doklady) Acad. Aci. U.R.S.S. **18**:603–606. 1938.
4. GREGORY, F. G., and PURVIS, O. N., Vernalization of winter rye during ripening. Nature **138**:973. 1936.
5. KIRICENKO, F. G., and BASSARSKAJA, M. A., On the presence of the third stage of development in wheat. Imp. Bur. Plant Genetics. Bull. 17. 1935.
6. KOSTJUCENKO, I. A., and ZARUBAILO, T. J., Vernalization of seed during ripening and its significance in practise. Herb. Rev. **5**:146–157. 1937.
7. KRAEVOI, S. J., and KIRICENKO, F. G., Dokl. Akad. Nauk S.S.S.R. **1**:171–176. 1935.
8. LOEHWING, W. F., Photoperiodic aspects of phasic development. Science **90**:552–555. 1939.
9. LYSENKO, T. D., Fundamental results of research on the vernalization of agricultural plants. Jarov. Bull. 4. 1932.
10. MCKINNEY, H. H., Vernalization and the growth-phase concept. Bot. Rev. **6**:25–47. 1940.
11. MCKINNEY, H. H., and SANDO, W. J., Earliness of sexual reproduction in wheat as influenced by temperature and light in relation to growth phases. Jour. Agr. Res. **51**:621–641. 1935.
12. MACKOV, F., SIMANSKII, M., and TRIGUBENKO, Zborn. Robit Agrofiziol. **1**:55–67. 1936.
13. VASILJEV, I. M., The response of certain agricultural crops to vernalization. Sovet. Agron. No. 6. 1939.
14. WHYTE, R. O., Phasic development of plants. Biol. Rev. **14**:51–87. 1939.
15. WHYTE, R. O., and OLJHOVIKOV, M. A., Photoperiodism in the plant kingdom. Nature **143**:301. 1939.
16. WORT, D. J., Vernalization of Marquis wheat and other spring cereals. BOT. GAZ. **101**:457–481. 1939.
17. WORT, D. J., Responses of various spring wheats to vernalization. Plant Physiol. **15**:137–141. 1940.

RESPONSES OF SUNFLOWER STEMS TO GROWTH-PROMOTING SUBSTANCES

JOHN L. BLUM

(WITH TEN FIGURES)

Introduction

Histological responses to different growth-promoting substances have been described recently for stems of a number of dicotyledons (2–11) and of three species of *Lilium* (1). It has been established that one such substance, indoleacetic acid, causes the various tissues to react in characteristically different ways in various species of plants. Since the previous investigations include work on responses in stems of several stelar types, but not many observations concerning the differences between the effects of the various substances,[1] it was thought desirable to compare the tissue responses of stems of a single species with what might be regarded as generalized and typical stem structure to a variety of different growth substances. The present study compares the effects of five growth-promoting substances, indole(3)acetic acid, indole(3)butyric acid, indole(3)propionic acid, naphthaleneacetic acid, and phenylacetic acid, on the stems of the sunflower, *Helianthus annuus* L. These acids were selected because they were readily available in crystalline form, and because they had been previously shown responsive in the *Avena* curvature test.

Material and methods

Sunflower plants grown under greenhouse conditions at various seasons of the year were used for the experiment. The work was carried on throughout 1938 and from January to May of 1939; during this period several different plantings were made, and the plants treated.

The plants were decapitated about 1 cm. above the third node, when the average length of the elongating third internode was somewhat more than 1 cm. for a group of contemporary plants. Treatment in the third internode usually was made 6–7 weeks after planting. A single decapitated plant was treated in one of three ways: (1) by smearing immediately the cut surface with lanolin containing 0.2 per cent growth-promoting substance, a comparatively weak concentration; (2) by smearing with pure lanolin; or (3) by no treatment after decapitation.

Material for histological work was fixed in formalin-acetic-alcohol fixative soon

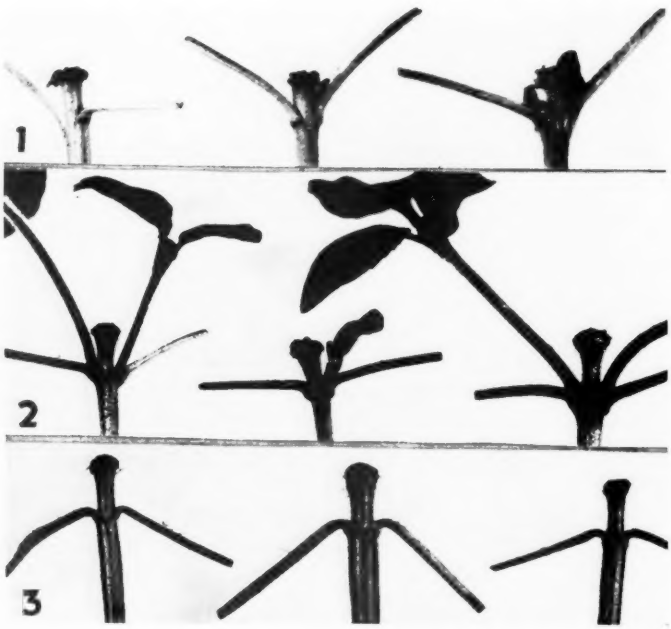
[1] A pertinent paper has appeared since this article was accepted for publication: MULLISON, W. R., Histological responses of bean plants to tetrahydrofurfuryl butyrate. BOT. GAZ. 102:373–381. 1940.

after treatment, and at various intervals up to 35 days. It was imbedded, sectioned, and stained by standard methods.

Observations

Gross responses

Within 24 hours after treatment, epinasty of the leaves at the third node is more or less evident for plants treated with indoleacetic (fig. 1) or with indolepro-



FIGS. 1–3.—Fig. 1, sunflower stems 14 days after treatment with indoleacetic acid above third node. Note irregular surface of callus and small axillary branches, development of which has been delayed owing to inhibiting effect of this acid. Blades of leaves removed for convenience in photographing. Fig. 2, 14 days after treatment with indolepropionic acid. Note epinasty of leaves, enlarged stump, and axillary branches. Development of latter inhibited only slightly. Fig. 3, 14 days after treatment with naphthaleneacetic acid. Note epinasty of leaves and lack of axillary branches, development of which has been completely inhibited.

pionic acid (fig. 2), and very conspicuous with naphthaleneacetic (fig. 3) and indolebutyric acid. Phenylacetic acid mixture induces little if any epinasty, even after several days. Epinastic bending of any one petiole does not increase markedly after a period of 1 day following treatment, and epinastic bending of leaves at the second node was never observed.

Treatment in the third internode does not occasion responses below the third node. Most of the responses (except for externally visible ones at the node) are confined to the callus and the upper 3–4 mm. of the decapitated stump of the stem from which, following application of the growth substance, the callus is formed. The region within 2–3 mm. above the node remains essentially unchanged from the time of decapitation and treatment. This permanent localization of the response in sunflower is of interest in light of work on the bean, which has been shown to respond, within 128 hours after treatment, as far as 5 cm. below the point of application (**7**).

Within 5–10 or more days after treatment the decapitated ends of the stems assume characteristic shapes, which are specific to a certain extent for each of the acids. Following application of indoleacetic, indolebutyric, naphthaleneacetic, and phenylacetic acid, growth is largely in the callus which develops above the former position of the cut surface, and the vertical sides of the decapitated stump remain approximately unchanged in outline. With indolepropionic acid there is an externally observable response below the cut surface, in that the upper portion of the stump swells considerably. In the uppermost 5–6 mm. of the stump, the amount of swelling as a result of application of this acid is about inversely proportional to the distance below the surface of decapitation, resulting in a stump which tapers from apex to base. The upper surface of a callus formed after the application of indoleacetic acid is frequently quite irregular; with the use of the other acids it is more commonly smooth.

Adventitious root tips appeared about 12 days after treatment at the outer edge of the apex of many of the tumors formed following treatment with indoleacetic acid. These roots never grew more than a few millimeters under greenhouse conditions. Although the sunflower forms such roots after indoleacetic acid has been applied, they are formed only occasionally after application with indolebutyric and indolepropionic acid, and never with naphthaleneacetic or phenylacetic acid. New roots were not formed to any extent after a period of 3 weeks following treatment with any of the acids.

Plants decapitated in the third internode and smeared with pure lanolin, or left untreated, form axillary branches at the third node, and sometimes the second node also, within 2 weeks. Certain of the acids inhibited development of the axillary buds from which such branches come. Counts have not been made on a large scale, but results from limited plantings indicate certain differences in the acids, in the extent to which they inhibit the development of axillary branches. Indoleacetic (fig. 1) and indolebutyric acid seem to inhibit bud development for a time, and any branches which develop appear relatively later or in fewer numbers than those produced by plants untreated or treated either with pure lanolin, indolepropionic acid (fig. 2), or phenylacetic acid. The latter substances appear to inhibit

bud development only slightly, if at all. Treatment with naphthaleneacetic acid causes complete inhibition of bud development (fig. 3). With one exception, plants treated with this acid did not produce axillary branches at any of the three nodes. This is in accord with the findings of MITCHELL and STEWART (10), who applied the same acid to decapitated stems of bean plants.

ANATOMY OF UNTREATED STEM

The sunflower stem as it appears normally (figs. 4, 5) is characterized by a cylinder of fifteen to thirty primary vascular bundles. Secondary thickening due to cambial activity is beginning or has begun at the time of treatment, and if the stem is allowed to grow normally, will develop a continuous vascular cylinder, interrupted at intervals by uniseriate rays. The large hairs borne on the epidermis may be one channel for conduction to the cortical cells of materials applied in lanolin, since it is difficult to smear the decapitated end of a stem and keep the numerous and sizeable hairs which subtend the cut surface entirely free of the mixture. The untreated stem is so generally familiar that detailed description is unnecessary.

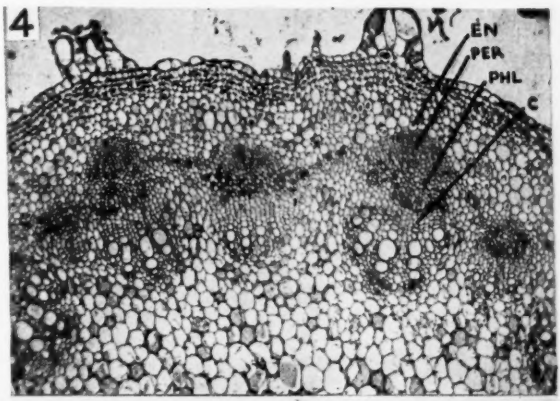
ANATOMY OF TREATED STEMS

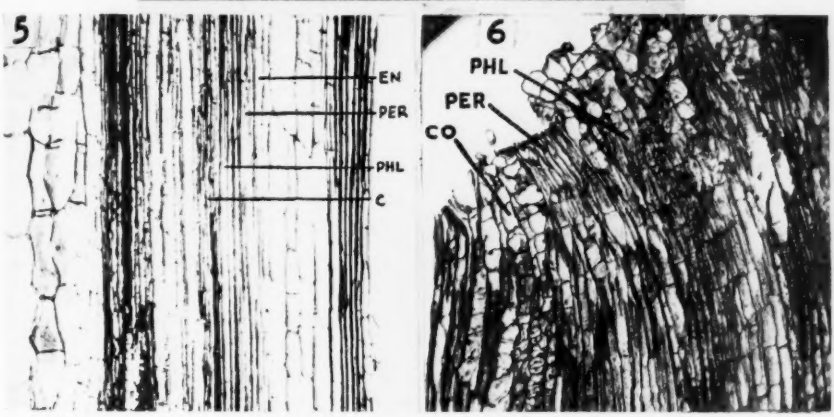
Sunflower stems which were decapitated and left untreated, or were smeared with pure lanolin, did not change materially in histological structure in the 3–4 weeks following treatment during which observations were made, except for shrinkage and loss of color and drying out of the exposed portions of those untreated. In such controls, secondary thickening and cell maturations were apparently suspended, and no appreciable callus tissue was formed. Other workers have shown that similar control stems of tomato (2), cabbage (3), and species of *Iresine* (6), *Lilium* (1), and *Mirabilis* (4), as in the sunflower, will not proliferate; but that those of bean will proliferate extensively and form considerable callus (7).

Certain of the parenchymatous tissues near the smeared surface, shortly after treatment with one of the substances, begin to proliferate. Cells of the tissues which are not responsive (for example, the epidermis, the pericycle, and the tracheids and vessels of the xylem) are not ordinarily broken and carried upward by this development but remain in their original position (fig. 6) and undergo some crushing. A single primary xylem vessel will thus appear to end blindly at its apical extremity in a mass of cells which have formed from proliferation in an apical direction of the xylem parenchyma around the vessel.

The pith is responsive to all the acids for some distance below the cut surface. The cells immediately below the cut surface at the time of decapitation divide and elongate rapidly, so that most of the callus tissue develops from the uppermost layers of cells. Divisions of cells within a few cell layers of the cut surface may not be followed by cell enlargement, with the result that a single pith cell will divide

and its daughter cells redivide many times, and all the daughter cells will occupy a region conforming in position and outline with that of the original parent cell. Other pith cells, or cells from the division of pith cells, mature into wound tra-





FIGS. 4–6.—Fig. 4, cross section in third internode of normal stem at time of treatment: *c*, cambium; *en*, endodermis; *per*, pericycle; *phl*, phloem. Fig. 5, longisection in third internode of normal stem at time of treatment. Fig. 6, longisection near apex of stem 4 days after treatment with indolebutyric acid: *co*, cortex; *per*, pericycle; *phl*, phloem. Note inactivity of pericycle and proliferation within phloem and cortex.

cheids. Such tracheids appear to bear no functional relation with one another, or they may be organized into short, bundle-like, vertically oriented groups, which end blindly at both ends in the parenchyma of the callus. Occasionally such tracheids connect with cells of the secondary xylem in the region of the ray.

Cells of the perimedullary zone or of the primary xylem parenchyma, in con-

trast to the pith, do not mature into such xylem cells, but by division and vertical elongation contribute to the formation of the callus.

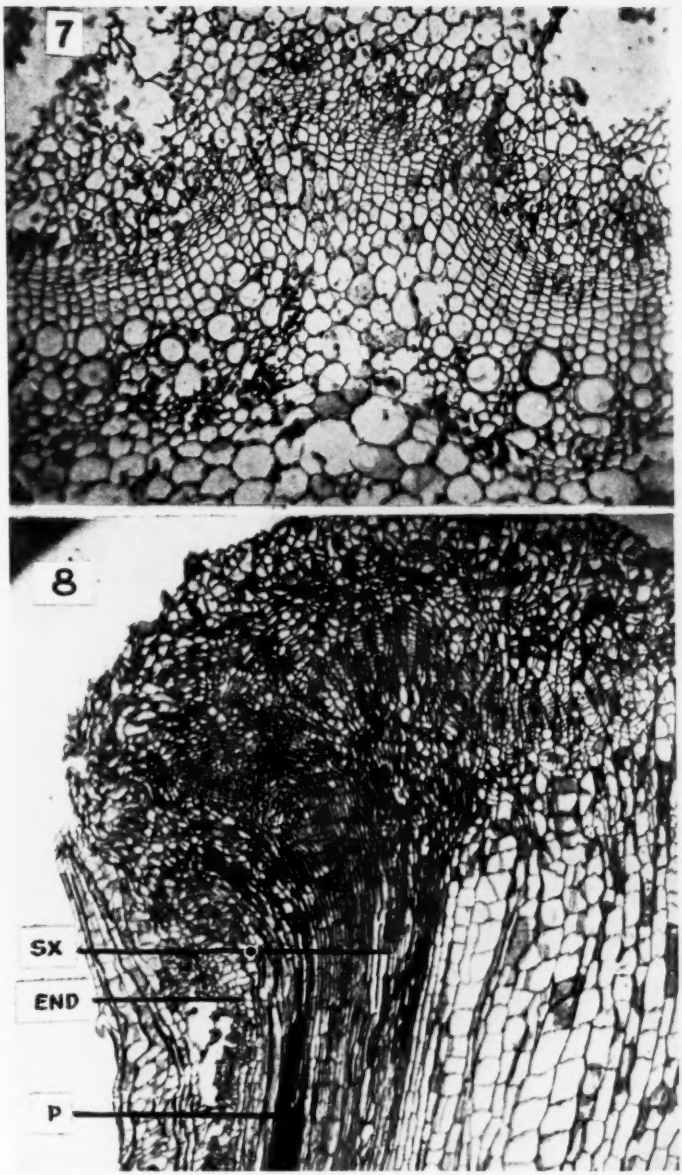
The cambium is highly reactive to naphthaleneacetic acid and to a less degree to all the other acids. In the proliferation of its cells nearest the cut surface, the cambium is believed to contribute to the developing callus, and its normal activity in secondary thickening is increased in the uppermost 3–4 mm. of the stump. With naphthaleneacetic acid there is a prominent increase in the formation of secondary xylem, the stump of the third internode becoming quite woody within 3–4 weeks after treatment. A similar response has been shown to follow treatment of other species of plants with naphthalene acetamide (**8, 10**). The degree of response in sunflower decreases from the cut surface down, so that the amount of secondary thickening 1–2 mm. above the node, if any, is about equal to that below the node, or to that of the third internode of an uncut and untreated control plant of comparable age.

Secondary thickening in normal plants, and in plants treated with any of the acids except phenylacetic, is more or less uniform for all regions upon the circumference of the cambium, and the cambium itself will appear in cross section as an approximate circle. With phenylacetic acid, however, there is greater cambial activity between the bundles than within them, resulting in a cambium cylinder which is vertically ridged in the region of the ray, and which appears in cross section for a few millimeters below the cut surface to be distended outward between two adjacent vascular bundles (fig. 7). This interfascicular cambial activity is not so great as that of the cambium after treatment with naphthaleneacetic acid, and it never causes the stem to become especially woody.

The parenchyma of the primary and secondary phloem is moderately reactive to all the acids (fig. 6). In stems treated with indoleacetic acid it contributes to the formation of the callus by cell division and elongation, and indirectly (through its derivatives in the callus) to the organization of adventitious root tips. The latter project externally from the callus near the cut surface. The development of such roots in other species of plants has been described in detail by previous workers, who have likewise traced the origin of the roots to the phloem parenchyma (**2, 3, 5**). All such roots do not develop entirely from the phloem in sunflower, and it has been indicated that comparable roots in *Pisum* may develop from a meristematic region which itself arises from derivatives of many cortical and stelar tissues (**11**).

The pericycle over the bundles, already thick walled at the time of treatment, has not been found appreciably responsive with any of the acids, either in cell division or in cell enlargement (fig. 6).

The endodermis is highly responsive for 1–2 mm. below the cut surface to at least naphthaleneacetic, indoleacetic, and indolebutyric acid, its cells reproducing by radial divisions and elongation rows of isodiametric or radially elongated cells



FIGS. 7, 8.—Fig. 7, cross section 0.5 mm. below upper exposed surface of callus in third internode of stem 18 days after treatment with phenylacetic acid. Note cambium distended outward between the two bundles. Fig. 8, longisection near apex of stump of stem 12 days after treatment with naphthaleneacetic acid: *end*, cells proliferated from endodermis; *p*, pericycle; *sx*, secondary xylem. Note lacunae in cortex.

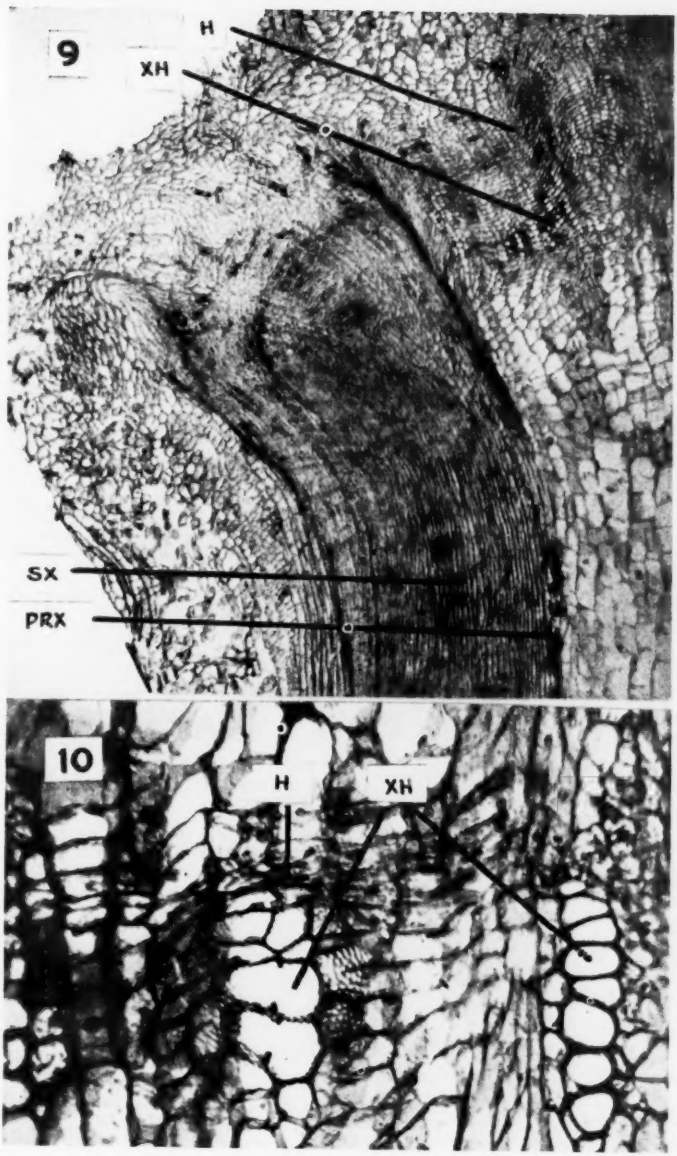
(fig. 8). Owing perhaps to unequal growth, individual rows become detached from those of rows of similar origin which are adjacent, and intercellular spaces are formed. These cells push against the outer cortical cells, forcing the latter (and hence the epidermis) outward, and in part account for any irregularity in the sides of the stump. Large lacunae develop in the cortex (fig. 8) following this rupture. Such lacunae may be due to disintegration of the middle lamella of certain of the cells, as suggested by SCOTT (11) for similar lacunae in auxin-treated stems of *Pisum sativum*. In the development of this endodermal mass of cells in sunflower, the casparian strips, when they can be traced, remain with the innermost cell of each radial row, so that the endodermis can be said to retain its identity. With indolepropionic acid there is considerable cell division in the cortex, but no lacunae have been observed.

The cortex external to the endodermis responds to any of the acids used by cell divisions and enlargements near the surface of application, and since the epidermis shows no response whatever, forms what is seen in cross section as the entire periphery of the callus. A characteristic cortical response is shown following treatment with indolepropionic acid, the cortical cells (and in part the pith) enlarging radially and evenly in proportion to their proximity to the surface of application to form the characteristic tapering stump just described, and illustrated in figure 2.

The formation of a corklike layer from the outermost cortical cells has been observed occasionally with the use of naphthaleneacetic acid. Sometimes this appears several millimeters below the cut surface.

In considering the responses shown in the cortex, it may be observed that, with indoleacetic acid, many of the adventitious root tips appear to be derived from meristematic cortical tissue alone. Development of these roots was not followed in detail, since they were rather infrequent in occurrence.

One activity evident within the stems in response to the application of growth-promoting substances has been set apart for discussion separately. This activity is the development of a horizontal cambium-like tissue (figs. 8–10) within the callus and in the plane of (or above) the cut surface, from cells which have proliferated from the xylem parenchyma and the pith. The horizontal "cambium" is continuous with the true cambium where the latter was cut across in decapitation, and it forms a platelike meristem over the surface of decapitation. It produces cells on its lower face which mature into xylem (figs. 9, 10) and on its upper face cells which mature into a tissue which is sometimes phloem-like in appearance. The immature xylem cells produced by this "cambial" activity elongate in a horizontal direction and become lignified with reticulate thickenings. The long axis of the cell after elongation may apparently be in any direction from the radial to the tangential. Thus there is formed across the stem, below the horizontal meristem, an unorganized mass of xylem cells in groups, all of which may be oriented in different directions. The horizontal cambium is not an entirely flat plate. It is more



FIGS. 9, 10.—Fig. 9, longisection at apex of stem 25 days after treatment with naphthaleneacetic acid: *h*, horizontal "cambium"; *prx*, primary xylem; *sx*, secondary xylem; *xh*, group of tracheids formed toward lower face of horizontal cambium. Note extreme development of secondary xylem, and lacunae in cortex. Fig. 10, longisection at cut surface of stem 14 days after treatment with indoleacetic acid: *h*, horizontal cambium; *xh*, groups of cells matured into tracheids after having been cut off toward lower face of horizontal cambium.

appropriately a plate whose circumference forms an approximately plane circle, but whose central portion is irregularly bent, warped, or dented (fig. 9). Its initials are developed successively, soon after treatment, in the cells formed from upward proliferation of the true cambium, the xylem parenchyma, and the pith. The initials can be traced internally over the cut ends of the xylem vessels several layers from the upper free surface of the callus. In early development the horizontal cambium lacks a central portion. The edges, approaching the center of the stem from all points on the circumference of the true cambium cylinder, may eventually close over the central region, perhaps uniting with previously isolated meristematic regions in the pith. The closing over in the center of the callus may or may not occur; when it does occur, it appears usually to be completed by about the twentieth day after treatment. Development of the horizontal cambium has been observed most conspicuously and most constantly with naphthaleneacetic acid; it has been observed less regularly following application with the other acids.

Discussion

These results point toward some degree of specificity for each of the various growth-promoting substances used in regard to the responses which result from their application. Following the application of indoleacetic acid, development of adventitious roots from the tumor was frequently observed. Such roots regularly were not formed in response to treatment with any of the other acids. Partial inhibition of the development of axillary branches at the third node occurs in response to treatment with this substance and to treatment with indolebutyric acid. Certain other responses following the application of indoleacetic are not specific for this acid, that is, similar responses occur after treatment with many or all of the other acids used.

Plants treated with indolebutyric acid have not been found to show distinctive responses and are distinguished from plants treated with the other acids on the basis of negative characters. The latter plants in all cases show responses which do not occur following the application of indolebutyric acid.

The typical response in stems which have been treated with indolepropionic acid is the growth in diameter of the apical end of the stem. This growth is due mostly to a characteristic enlargement of cells of the cortex and pith, the enlargement being preceded by divisions of cells only 1–2 mm. below the cut surface. Application of indolepropionic acid will inhibit the development of axillary buds only slightly, and in this respect also its effects can be distinguished from those of the other substances used.

Following treatment with naphthaleneacetic acid marked responses occur. Epinasty of the leaves at the third node is usually strongly evident within 24 hours after application. Cambial activity is stimulated by this substance more than by any of the other four used, and the stump becomes relatively woody in a short

time. The development and activity of the horizontal "cambium" is also markedly stimulated. One of the most sharply distinguishing responses to this treatment is the complete inhibition of bud development in the axils of the leaves at the third node.

The only response characteristic and specific for plants treated with phenylacetic acid is the stimulation of the activity of the interfascicular cambium, this activity resulting in a cambium cylinder which is distended outward between the vascular bundles.

It is to be emphasized that specific responses of an individual stem to treatment with any of these acids can never be predicted with certainty. The responses have been found usually to occur as described, so that a group of stems which have responded to treatment with indolepropionic acid, for example, can be identified and distinguished as such from groups of stems treated with any of the other four acids. But it is possible to select from the group atypical individuals which, taken alone, might appear both externally and histologically more like an indoleacetic tumor, or a phenylacetic tumor, or a composite of these, than like other members of its own group. There has been also considerable variation in the time elapsing between treatment and response, and in the degree of final response. It would appear, therefore, that a study based upon averages over a very large population of treated plants should be made of such responses. The present study has been concerned with the late or final effect of the stimulants applied, rather than with the development of these effects in detail over the period of time between treatment and completion of the experiment.

Summary

1. Sunflower plants were treated upon a decapitated surface in the third internode with one of five growth-promoting substances in lanolin.

2. Certain responses are common to treatment with all of the acids. Among these responses are: the formation of parenchymatous or meristematic callus tissue at the exposed surface of the pith, of the parenchyma of the xylem and phloem, and of the cortex, especially the endodermis; and increased secondary thickening near the apex of the stump. The conducting elements of the xylem and phloem, the pericycle, and the epidermis do not react to any of the acids.

3. A horizontal cambium-like tissue is formed soon after treatment, which connects with the true cambium at the cut edges of the latter and forms a platelike meristem across and within the callus. Cells which it forms to the lower side develop into tracheids.

4. Some of the acids bring about characteristic and specific responses, as follows:

a. Indoleacetic acid causes the partial inhibition of bud development at the third node and the formation of adventitious roots.

b. Indolebutyric acid causes partial inhibition of bud development, but it does not cause general formation of root tips, extreme secondary thickening, marked cell enlargement, nor marked activity of the interfascicular cambium.

c. Indolepropionic acid inhibits bud development slightly if at all, and its application results in a characteristic stump which tapers from apex to base, owing to a gradient of cell enlargement, chiefly in the cortex and pith, and near the uppermost portion of the tumor to cell divisions.

d. Naphthaleneacetic acid causes marked epinasty, completely inhibits bud development, causes extreme development and differentiation of secondary xylem, and marked development and activity of the horizontal "cambium."

e. Phenylacetic acid induces in the third internode disproportionate activity of the interfascicular cambium, the cambium becoming outwardly distended between the vascular bundles.

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LITERATURE CITED

1. BEAL, J. M., Histological responses of three species of *Lilium* to indoleacetic acid. BOT. GAZ. **99**:881–911. 1938.
2. BORTHWICK, H. A., HAMNER, K. C., and PARKER, M. W., Histological and microchemical studies of the reactions of tomato plants to indoleacetic acid. BOT. GAZ. **98**:491–519. 1937.
3. GOLDBERG, ETHEL, Histological responses of cabbage plants grown at different levels of nitrogen nutrition to indole(3)acetic acid. BOT. GAZ. **100**:347–369. 1938.
4. HAMNER, K. C., Histological responses of *Mirabilis jalapa* to indoleacetic acid. BOT. GAZ. **99**:912–954. 1938.
5. HAMNER, K. C., and KRAUS, E. J., Histological reactions of bean plants to growth promoting substances. BOT. GAZ. **98**:735–807. 1937.
6. HARRISON, B. F., Histological responses of *Iresine lindenii* to indoleacetic acid. BOT. GAZ. **99**:301–338. 1937.
7. KRAUS, E. J., BROWN, NELLIE A., and HAMNER, K. C., Histological reactions of bean plants to indoleacetic acid. BOT. GAZ. **98**:370–420. 1936.
8. KRAUS, E. J., and MITCHELL, J. W., Histological and physiological responses of bean plants to alpha naphthalene acetamide. BOT. GAZ. **101**:204–225. 1939.
9. LINDNER, R. C., Effects of indoleacetic and naphthylacetic acids on development of buds and roots in horseradish. BOT. GAZ. **100**:500–527. 1939.
10. MITCHELL, J. W., and STEWART, W. S., Comparison of growth responses induced in plants by naphthalene acetamide and naphthalene acetic acid. BOT. GAZ. **101**:410–427. 1939.
11. SCOTT, FLORA M., Anatomy of auxin treated etiolated seedlings of *Pisum sativum*. BOT. GAZ. **100**:167–185. 1938.

FLORAL ANATOMY AND MORPHOLOGY OF ANEMOPSIS CALIFORNICA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 524

CHARLES H. QUIBELL

(WITH THIRTY-EIGHT FIGURES)

Introduction

This paper describes the vascular supply to and in the individual flower in the inflorescence and also the development of the ovule and megagametophyte. *Anemopsis californica* (Nutt.) H. and A. is a moisture demanding, alkali tolerant, perennial herb of the southwestern United States and northern Mexico, for which a new variety subglabra has been recently established by KELSO (7). The material used here is of that variety.

Anemopsis is a member of the Saururaceae, frequently included as a tribe in the Piperaceae. On the basis of anatomical considerations, ROUSSEAU (8) has reviewed the systematic treatment of the Piperales and concludes that *Saururus* is more closely related to *Piper* than the latter is to *Peperomia* of the Piperaceae. HOLM (3) has described the vegetative morphology and anatomy of the species, including the anatomy of the involucral and floral bracts. SCHMITZ (9) has described the development of the flower and vascular anatomy of several species of the Piperales but not of *Anemopsis*. JOHNSON (6), in a paper dealing with the relationships of the order, has reviewed the development of four genera already investigated and added data on four more, including *Anemopsis*. Of this he reports that "the functional megaspore is one out of two potential megaspores. . . . .A tapetum is formed and is persistent. . . . .The mature embryo-sac is a typical seven-nucleate one. . . . .The first division of the endosperm nucleus is followed by a cell wall cutting the embryo-sac into an upper and a lower cell . . . . it is the upper one of these two primary cells that divides further to form a considerable mass of endosperm. The lower cell forms an elongated flask-shaped haustorium with but a single nucleus." JOHNSON does not elaborate these statements and his paper is not illustrated. HÄUSER (2) interprets JOHNSON's statement with regard to two megaspores to mean that after the first division of the megaspore mother cell only the lower nucleus divides again and the megagametophyte is derived from the two megaspores resulting from such division. On the basis of the same report, SCHNARF (11) assumes tentatively that the megagametophytic development is of the *Scilla* type. The observations reported here confirm SCHNARF's assumption. A 16-nucleate megagametophyte derived from four megaspores has

been reported for *Peperomia pellucida* (1, 5). The investigations stimulated by this discovery have been summarized by SCHNARF (10, 11). Although all the seventeen species of *Peperomia* which have been studied possess 16-nucleate megagametophytes, and other 16-nucleate megagametophytes have been discovered in several unrelated families, no other genus in the Piperales exhibits megagametophytes with more than the usual number of eight nuclei. These megagametophytes are derived, in different genera, from one, two, or four megaspores.

Material and methods

The material was collected at various stations near Fresno, but chiefly in a meadow along Kings River above Centerville, California. The inflorescences, or more frequently sections of them, were killed and fixed in a variety of fluids, of which an alcohol-formalin-acetic acid mixture proved most satisfactory. Heidenhain's haematoxylin-orange G combination was satisfactory.

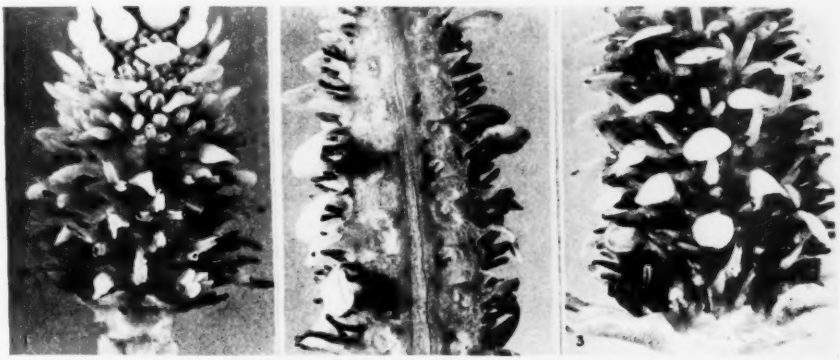
Observations

INFLORESCENCE AND FLOWER.—The thick horizontal rhizome is perennial, a rosette of leaves expanding from its apical bud each spring. From the axils of some of these leaves arise long stolons. Vegetative propagation seems to be the chief means of distribution, few seedlings having been observed. From the axils of other leaves rise single flowering stems whose apices are at first surrounded by the single cauline bract borne on these stems. The cylindrical or slightly conical primary inflorescence, pushed up out of this enveloping bract by the growth of its peduncle, is then protected by the whorl of 6–8 white involucral bracts which are turned up over it. When these spread out horizontally, the inflorescence is still covered for a short time by the small floral bracts, one subtending each of the numerous, spirally arranged flowers, except the basal ones just above the involucral bracts. The horizontal spreading of these bracts is acropetal (fig. 1).

The individual flowers, when thus uncovered, for a brief period show only the closely packed, turgid tips of the six yellow anthers. Subsequent growth spreads the anthers, uncovering three grooved stigmas, whose growth then brings their tips well above the anthers (fig. 1). The ovary has a single locule and three parietal placentae. It is completely inferior, so that the stigmas and the nearly sessile anthers project from the general surface of the inflorescence axis (fig. 2). Each of the three stigmas is grooved the full length of its inner face. These three grooves are united at the surface of the inflorescence axis to form an actual or potential pore leading into the locule. According to JEPSON (4) there are 6–10 ovules on each placenta or 18–30 ovules per flower. Counts of thirty flowers (excluding those near the apex of the inflorescence) distributed among six heads averaged 8.5 ovules per flower. The arrangement of two stamens opposite each carpel is shown in

figures 1 and 3. Two or three leaves arise from a meristem in the axil of the cauline bract and sometimes one or two secondary inflorescences arise in their axils.

VASCULAR SUPPLY OF INDIVIDUAL FLOWER.—The vascular bundles of the axis of the inflorescence are arranged in an irregular ring. The vascular supply of the individual flower is a single bundle (fig. 4 *X*) which, at the level of the next flower directly below, swings outward from the ring of bundles, rises parallel to it until the base of the flower is reached, and then is divided into seven bundles, all of which are turned outward around the locule (figs. 5–8). At a point level with the top of the locule six of these seven bundles undergo a series of divergences and



FIGS. 1–3.—Fig. 1, lower three-fourths of inflorescence. Fig. 2, longisection showing ovaries surrounded by inflorescence axis. Fig. 3, similar to and somewhat older than inflorescence in fig. 1.

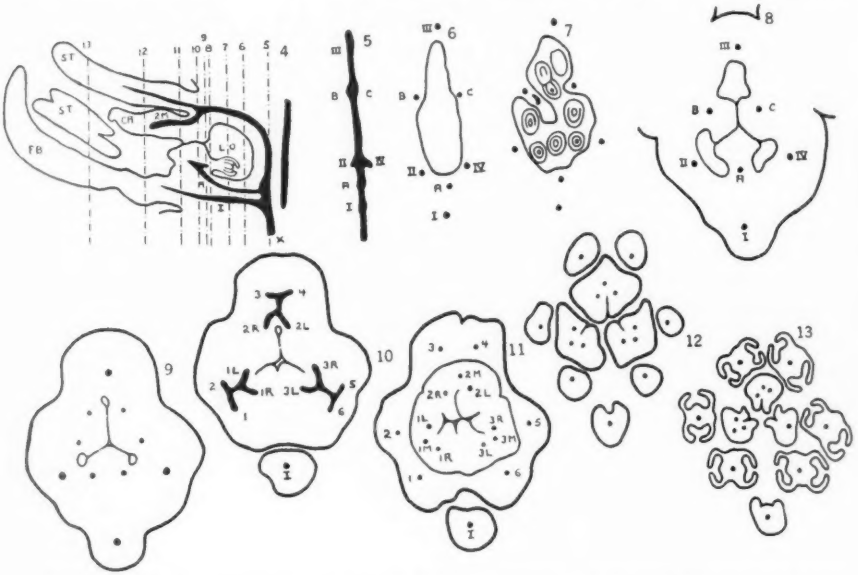
anastomoses (figs. 4, 9–11) which result in fifteen bundles supplying the filaments and styles (figs. 12, 13).

The bundle of the bract (*I*) is the first divergence from the main supply bundle, and without anastomosing with any other it provides the entire vascular supply for that organ. The lower placental bundle (*A*) is the next divergence, followed in turn by two paired divergences, the lower stamen bundles (*II*, *IV*) and the upper placental bundles (*B*, *C*), and by the upper stamen bundle (*III*) which extends out over the upper side of the locule.

The placentae are parietal and extend little more than half way down the locule wall (fig. 2). Above them (level with the top of the locule) the placental bundles split in two, diverge right and left at sharp angles, and then ascend as lateral bundles in the two corresponding styles. The stamen bundles, one opposite each carpel, extend around the locule to a point somewhat above its top, are split trichotomously, and diverge at sharp angles. One branch extends inward and is the central bundle in the style, while the other two spread right and left and extend into the two stamens associated with the carpel. The central bundle of each style,

at the point where it is turned upward into it, is usually connected by horizontal branches with the two stylar laterals.

There are occasional variations from this pattern. The basal flowers in the inflorescence lack floral bracts. No instance of variation in the number or arrangement of the six placental and stamen bundles has been observed, but variations in the fifteen divergences from them are comparatively frequent. A relatively large proportion of flowers lack one or more stamens or a style. Short abortive



FIGS. 4–13.—Fig. 4, longisection of flower bud in inflorescence still inclosed by involucral bracts. Figs. 5–13, cross sections of flower in bloom, at levels corresponding to those indicated by numbered lines on fig. 4. *FB*, floral bract; *ST*, stamen; *CA*, upper end of carpel; *LO*, locule and one ovule; *I*, floral bract bundle; *II*, *III*, *IV*, stamen supply bundles; *A*, *B*, *C*, placental bundles; *1*, *2*, *3*, *4*, *5*, *6*, stamen bundles; *1R*, *1M*, *1L*, *2R*, *2M*, etc., right middle, and left stylar bundles of carpels *1*, *2*, and *3*.

styles, lacking stigmas, have their middle bundles diverged from the corresponding stamen bundle, but they do not have lateral bundles, which if diverged at all from the corresponding placental bundles form only short vestigial traces which do not extend into the style base. All instances of abortive stamens showed at least some anther development and vascular bundles. Possibly those abortive stamens which consist of a short sharp-tipped filament, with no vestige of anther development, would have no vascular bundle.

A doubling of all the lateral stylar bundles was observed in one of the three flowers examined in each of two inflorescences. The extra bundles were short,

derived by splitting of the divergences from the placental bundles, and terminated at about the level of the anastomoses between the main and lateral bundles in or just below the stylar base. They are not involved in this anastomosis. The anastomoses between main and lateral stylar bundles are frequently incomplete, but in no flower observed were they absent in all styles. Occasionally a placental bundle is diverged into three instead of two, and the extra one is connected with the base of a middle stylar bundle.

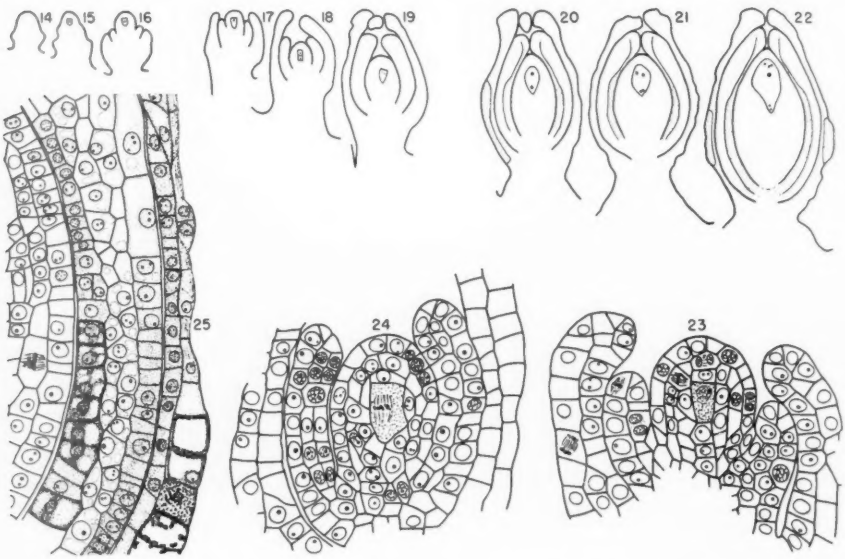
INTEGUMENTS.—The outer integument arises first, followed so quickly by the inner that the latter for a brief period may overgrow the former (figs. 14–16). Until the four megaspore nuclei have been formed, the growth rate of the outer integument exceeds that of the nucellus, so that a large space is developed above the latter (figs. 17, 18). As a result of change in relative growth rates during the prolonged 2-nucleate phase of the megagametophyte, this space is occupied by the nucellus and the rim of the inner integument (figs. 19, 20), and subsequently the growth rates of the integuments and nucellus keep pace with each other (figs. 21, 22). Both integuments are two cell layers in thickness in the beginning (fig. 23). In the outer integument the number usually does not increase except at its base and apex, when development of the embryo is well advanced. In the inner integument a third layer results from periclinal divisions in the inner layer, beginning about the time the megaspore mother cell divides (fig. 24). A fourth layer is more or less completely formed owing to doubling of the middle layer, when the megagametophyte is 8-nucleate (fig. 25). The largest cells of the ovule occur in the outer layer of the inner integument opposite to and above the megagametophyte. These cells are three or more times as long as broad, even as early as the end of the 2-nucleate phase (figs. 20, 25). During the latter half of this phase the cells of the inner layer, opposite and below the megagametophyte, grow much larger in both dimensions than those in the upper part. Beginning in isolated cells, the protoplasts of this portion of the layer all take a deep stain by the end of the 4-nucleate phase. In post-fertilization stages even the thinner portion of the layer over the top of the nucellus stains in this manner (figs. 20–22, 25).

In the outer integument the cells of the outer layer become much shrunken and stain weakly, so that their end walls are difficult to distinguish; but there are occasional short vertical rows of much enlarged cells which protrude from the surface of the ovule and later stain deeply like those of the inner layer of the inner integument (figs. 20, 22, 25).

NUCELLUS.—The apex of the nucellus, above the point of origin of the inner integument, is at first small and rounded (fig. 15), but by the time the rim of the outer integument passes beyond this apex the median longitudinal section of the nucellus is a truncated oval or ellipse (fig. 17). It shows little subsequent change in size or shape until after the megaspore mother cell divides, although in the

meantime the integuments and the ovule base have grown considerably (figs. 17, 18). During the 2-nucleate phase of the megagametophyte the shape of the nucellus, in longitudinal section, becomes a considerably less truncated ellipse (figs. 18–20). Its growth in length is most pronounced below the megagametophyte, with the result that the latter exchanges its original central position for one in the upper half of the nucellus.

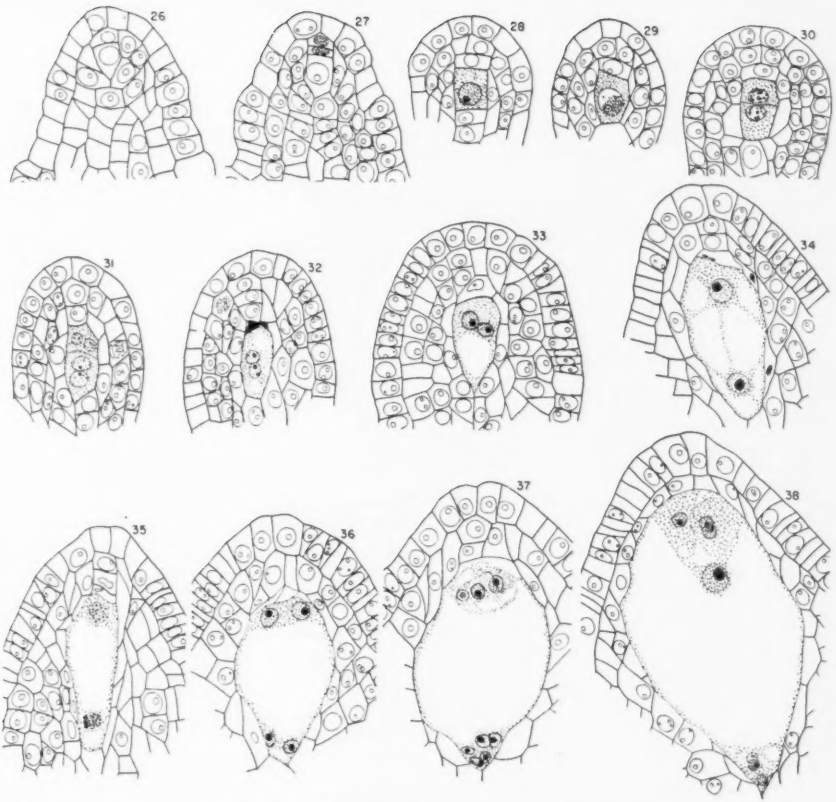
The megaspore mother cell and two parietal cells arise from a hypodermal primary sporogenous cell. These three cells are recognizable in some ovules when



FIGS. 14–25.—Successive stages in ovule development. Megaspore mother cell present in fig. 15 and in prophase of meiosis in figs. 16 and 17; first meiotic division complete in fig. 18; 2-nucleate stages of megagametophyte in figs. 19 and 20, 4- and 7-nucleate stages in figs. 21 and 22. Figs. 23–25, stages in integument development from prophase of meiosis (fig. 23) to 8-nucleate phase of megagametophyte (fig. 25).

the inner integument is initiated (figs. 26, 27). During the prophase of the first meiotic division the outer parietal cell frequently undergoes anticlinal division. A periclinal division of this cell or any division of the inner parietal is rare (figs. 23, 24, 28, 29). Subsequent encroachment of the 2-nucleate megagametophyte upon the parietal cells is steadily balanced by their growth and division tangentially until the megagametophyte nuclei divide, after which frequently only one nucellar cell intervenes between megagametophyte and epidermis (figs. 32–38).

MEGASPORE MOTHER CELL.—This cell begins the protracted prophase of the



FIGS. 26–38.—Fig. 26, megaspore mother cell and primary parietal cell; inner integument just starting. Fig. 27, primary parietal cell divided; megaspore mother cell still differentiated only slightly, except in size. Fig. 28, megaspore mother cell in prophase of meiosis. Fig. 29, same in synizesis. Fig. 30, first meiotic division completed. Fig. 31, four megaspores in two cells after considerable growth has occurred in chalazal pair. Fig. 32, late stage in degeneration of micropylar megaspores. Fig. 33, 2-nucleate megagametophyte following resorption of micropylar megaspores. Fig. 34, nuclei at poles and in position for division; megagametophyte and nuclei slightly larger than average. Fig. 35, anaphase of division in 2-nucleate megagametophyte; axis of spindles at right angles to that of megagametophyte and to each other; chalazal division figure complete; one set of chromosomes of micropylar figure present in next section. Fig. 36, 4-nucleate megagametophyte. Fig. 37, polar nuclei about to fuse; egg to left. Fig. 38, 7-nucleate megagametophyte; egg nearer to observer than synergid, and its cytoplasm very diffuse. Antipodals represent extreme of preservation for this stage. Upper antipodal drawn in from second section.

first meiotic division before the rim of the outer integument passes beyond that of the inner (figs. 16, 28). The axis of the spindle for this division in the cases observed is parallel to the long axis of the ovule (fig. 24). Of the resulting cells the chalazal one is usually slightly larger (fig. 30). Division of the two daughter nuclei follows quickly. In two of the four instances observed there is a slight lag in the division of the micropylar nucleus, while in the others division is simultaneous. In all the spindles the axes were either oblique or across the cells and at various angles with each other. Walls were not formed, nor was there indication of a cell plate. The result is four megaspore nuclei in two cells,—the chalazal one, as subsequent events show, being the beginning of the 2-nucleate phase of the megagametophyte (fig. 31).

MEGAGAMETOPHYTE.—Marked size differences between the two pairs of nuclei become apparent quickly, since the micropylar nuclei do not grow following division, while the chalazal pair does (fig. 31). Growth of the megagametophyte begins soon after disappearance of the spindles, and in its first phase is entirely at the expense of the micropylar cell and the two megaspore nuclei it contains, so that at the end of this phase the megagametophyte has attained the size and shape of the megaspore mother cell at its initial division (figs. 30–32). The nuclei in the micropylar cell do not change in their staining reactions until at, or just before, their obvious structural degeneration begins. When this occurs the protoplast shrinks, with the cytoplasm clinging to the wall at several points. As the cell is further crushed, the nuclei become somewhat smaller. Then the cell and its contents are reduced to a dark staining cap and finally completely resorbed (figs. 32, 33).

The two nuclei of the megagametophyte lie close together almost as soon as formed, one above the other, at or near the center of the cell. Vacuoles appear above and below the nuclei without disturbing their position (figs. 31, 32). With complete disappearance of the micropylar cell, the nuclei frequently are shifted to a side-by-side position, but in the majority of instances the two vacuoles persist. When there is only one vacuole it may occupy either end of the megagametophyte (fig. 33). The megagametophyte grows steadily at the expense of the nucellus, until it has become about double the dimensions of the megaspore mother cell. The nuclei then move apart and take up positions nearer the poles, with a vacuole between them (fig. 34). Division follows quickly, and in the three instances observed the angles of the spindle axes with the axis of the ovule and with each other vary (fig. 35).

The 4-nucleate phase of the megagametophyte is relatively brief (fig. 36). In some inflorescences the chalazal end of the megagametophytes extends down nearly to the midpoint of the nucellus, while in others it reaches no farther than a fourth or a third the nucellar length. The four megagametophyte nuclei are in two

pairs, each pair imbedded in a dense, dome-shaped mass of cytoplasm at opposite ends of a large central vacuole. When first formed all four nuclei are equal in size, but while there is considerable increase in the diameters of the micropylar pair the chalazal pair does not grow (fig. 36). Division is simultaneous in the three examples observed; and in one 4-nucleate megagametophyte whose nuclei were just about to pass into the metaphase and in three very young 8-nucleate megagametophytes, all the nuclei are in the same stage. The 8-nucleate phase at first reflects the situation of the 4-nucleate phase regarding the size differences of the nuclei and the rather dense and restricted cytoplasmic masses at the poles. Each nucleus quickly organizes its mass of cytoplasm, in which there begins a characteristic type of differentiation. In the chalazal group the cytoplasm often becomes located chiefly at the sides of the nucleus and is somewhat strung out. The chalazal polar nucleus becomes readily distinguishable when the three antipodal nuclei shrink and are more chromatic, while it at first shows little change in size or appearance. Since degeneration of the antipodals is sometimes delayed or is not simultaneous, there are instances in which the chalazal polar nucleus cannot be definitely identified for a time.

The four micropylar nuclei are easily distinguished by the time the chalazal polar nucleus can be recognized with certainty. The polar nucleus derived from the micropylar end is always slightly lower down in the group relative to the vertical axis of the megagametophyte, but it is never quite separated from the others. The positions of the egg and synergid nuclei relative to the longitudinal axis of the megagametophyte are variable, but the synergid nuclei are readily recognized by their large and rather dense masses of cytoplasm, which occupy—or shortly come to occupy—the major portion of the micropylar end of the megagametophyte and become less dense or vacuolate at the chalazal margins. The egg nucleus, crowded against the side, has a much smaller mass of cytoplasm in which a very large vacuole usually develops, either laterally or toward the micropyle. Its nucleole is usually appreciably smaller than those of the synergid nuclei (fig. 37).

Before the polar nuclei fuse the chalazal one has increased in size to equal that of the micropylar (fig. 37). Fusion always occurs in the chalazal half of the megagametophyte, usually rather close to the antipodals. The endosperm nucleus is large and sometimes migrates to the micropylar end of the megagametophyte (fig. 38). By this time, the egg apparatus is well differentiated. The antipodals have usually degenerated considerably; one or more of them may have disappeared completely. Figure 38 illustrates the extreme of preservation of antipodals at this stage; more often the cytoplasm of those persisting has collapsed against the chalazal end of the megagametophyte, and the nuclear outlines—or even the nucleoles—are difficult or impossible to distinguish. Fertilization has not been observed.

Summary

1. The flower of *Anemopsis californica*, subtended by a small floral bract, consists of six stamens, three carpels, and an ovary completely buried in the inflorescence axis.

2. The vascular supply of the individual flower and its bract originates in a single bundle of the ring of bundles in the inflorescence axis. This bundle splits up into seven: the bract bundle and three stamen bundles alternating with three placental ones.

3. Above the ovary the stamen and placental bundles branch and anastomose in such a way as to provide one bundle for each stamen and three for each style.

4. The ovule is orthotropous, with two integuments and an eventually massive nucellus. The outer integument is initiated first. Both integuments exhibit certain differentiations in their cell layers.

5. The megaspore mother cell, which with two parietals arises from a hypodermal cell, divides twice, but a wall is formed only after the first division.

6. The pair of megaspores in the upper cell degenerate and the megametophyte is formed after two more nuclear divisions of the two megaspores in the lower cell.

7. The polar nuclei fuse in the chalazal end of the megagametophyte before fertilization.

8. The antipodals have usually disappeared or are degenerating before fertilization.

The subject of this investigation was suggested by Dr. G. M. SMITH of Stanford University. Encouragement and direction were given during the progress of the work by members of the department of botany of the University of Chicago.

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LITERATURE CITED

1. CAMPBELL, D. H., A peculiar embryo sac in *Peperomia pellucida*. Ann. Bot. **13**:626. 1899.
2. HÄUSER, ROBERT, Untersuchungen an Makrogametophyten der Piperaceen. Beitr. Allg. Bot. **1**:115–140. 1916.
3. HOLM, THEO, *Anemopsis californica* (Nutt.) H. and A. An anatomical study. Amer. Jour. Sci. **169**:76–82. 1905.
4. JEPSON, W. L., A manual of the flowering plants of California. Berkeley. 1925.
5. JOHNSON, D. S., On the endosperm and embryo sac of *Peperomia pellucida*. BOT. GAZ. **30**:1–11. 1900.
6. ———, Seed development in the Piperales and its bearing on the relationships of the order. Johns Hopkins Univ. Circ. N.S., no. 5. (no. 178) 1905.
7. KELSO, LEON, A note on *Anemopsis californica*. Amer. Midl. Nat. **13**:110–113. 1932.
8. ROUSSEAU, D., Contribution à l'anatomie comparée des Piperacées. Acad. Roy. Sci. Belg., Ser. 2. **9**:1–45. 1927.
9. SCHMITZ, FR., Die Blüthen-entwicklung der Piperaceen. Bot. Abhand. Hanstein **2**:1–74. 1872.
10. SCHNARF, K., Embryologie der Angiospermen. Berlin. 1927.
11. ———, Vergleichende Embryologie der Angiospermen. Berlin. 1931.

REINVESTIGATION OF POLYSOMATY IN SPINACIA

CHARLES A. BERGER

(WITH EIGHTEEN FIGURES)

Introduction

Root tips of *Spinacia oleracea* and some other plants contain, in addition to diploid cells with the $2n$ number of chromosomes, many polyploid cells with $4n$, $8n$, and rarely $16n$ chromosomes. This phenomenon, termed polysomaty by LANGLET (**13**), is not the result of artificial treatment of any kind but is a regular and constantly occurring process of development. The polyploid metaphases are of two kinds (figs. 11–16), some having their chromosomes in closely associated pairs, others with unpaired chromosomes. Since its discovery by STOMPS (**20**) in 1910, a number of papers (**14, 13, 21, 22, 16**) have appeared describing the phenomenon in *Spinacia* and attempting to account for the origin of the polyploid cells. The phenomenon has been reported in other plants also (**15, 13, 4, 5, 17, 11, 12, 6**).

Considerable difference of opinion exists, not only regarding the origin of polysomaty but also in the description of the phenomenon itself. In the larval ileum of *Culex pipiens* and other mosquitoes, a similar developmental process occurs involving the formation of cells of different degrees of polyploidy in the same tissue. Here it has been shown (**1, 2**) that the polyploid condition, ranging from $2n$ to $64n$, is the result of successive reproductions of the chromosomes within the resting nucleus. Two years ago a reinvestigation of the situation in *Spinacia* was begun to ascertain whether the polyploid cells may not arise in the same manner as those of *Culex*, by chromosome reproduction within the resting nucleus. At that time no one had yet proposed this solution, previous investigators having suggested that polysomaty was due to nuclear fusion or a double prophase "splitting" of chromosomes. When the present investigation had been in progress for more than a year, two workers in Sweden (**7**) published convincing evidence that the polyploid condition was indeed due to successive chromosome reproduction in the resting stage. They also pointed out the similarity of the process to that reported in *Culex*. Their paper and the earlier publication of LORZ (**16**) summarize the preceding work, and each adds valuable contributions to the discussion. Accordingly the present paper will be restricted to controversial points on which new evidence is available.

Material and methods

Seeds of *Spinacia oleracea*, variety Old Dominion, were obtained from Dr. F. S. HOLMES of the seed laboratory of the University of Maryland Agricultural Experi-

ment Station. They were germinated on filter paper in a moist chamber. At the time the tips were removed the total length of the root was measured and recorded. The Craf fixative was used, a modification by RANDOLF (19) of the chromo-aceto-formalin mixture. For nearly all purposes of this investigation only cross sections were of value. These were cut at 10μ, and all the sections of the first 1.5 mm. were mounted so that it was possible to determine position easily by counting the sections. The preparations were stained with iodine gentian-violet orange G.

Observations

NEW EVIDENCE ON DISPUTED POINTS

There is some diversity of opinion as to the tissues in which polysomaty occurs. All workers have found polyploid cells in the periblem of root tips, and some report it from other tissues as well. In *Cannabis sativa* four investigators give different results. LANGLET (13) found polysomaty in the periblem, plerome, dermatogen, and calyptrogen; DE LITARDIÈRE (15) and MEURMAN (17) found it mostly in the periblem with a few polyploid cells in the plerome; BRESLAWETZ (5) found it exclusively in the periblem. In the case of *Spinacia* there is more agreement. LORZ (16) found many polyploid cells in the periblem and one example in the plerome; GENTCHEFF and GUSTAFSSON (7) found them only in the periblem. In the work here reported polyploid cells were found only in the periblem. The work on *Cannabis*, and that of MEURMAN (17) on *Acer*, show that in other species of plants the phenomenon is not restricted to the periblem but may occur in other tissues of the root tip. Nor is the phenomenon restricted to root tips. ERVIN (6) found it in the stem tip and leaf primordia of *Cucumis melo* as well as in the roots. It is possible that this will be found to be true of *Spinacia* and other plants showing polysomaty in their root tips.

SEQUENCE OF POLYPLOID FORMS

By sequence is here meant the relative positions in the root where the different degrees of polyploidy occur. It is indicated by the distance in microns from the extreme tip of the root cap. LORZ (16) gives the following order: $2n$–$4n$ paired; $4n$–$8n$ paired; $8n$–$16n$ paired. GENTCHEFF and GUSTAFSSON (7) differ from this on one point. In thirteen out of seventeen roots they found unpaired tetraploid metaphases before the paired, that is, nearer the tip. On this point the observations here recorded agree with those of LORZ. In only two out of sixteen root tips thoroughly studied (table 1) did unpaired tetraploid metaphases appear nearer to the tip than paired tetraploids. In one of these roots, no. 28, the number of polyploid metaphases was so small that their relative position is of little significance. The other root, no. 29, was relatively old, being 55 mm. in length, so it is reasonable to assume that the unpaired tetraploids had already undergone a division, in

which the chromosomes were paired, since the beginning of germination. The actual distances in microns as given by GENTCHEFF and GUSTAFSSON (7) are as follows (figures represent mean from the measurement of six roots): first $2n$ metaphase in periblem at 240μ, first $4n$ paired at 430μ, first $4n$ unpaired at 370μ, first $8n$ paired at 480μ, first $8n$ unpaired at 640μ, and first $16n$ paired at 870μ.

GENTCHEFF and GUSTAFSSON divided their roots into four groups—"the first consisting of roots just beginning to germinate, thus showing only a few divisions. The other groups depict the increasing germinating process." This realization

TABLE 1

DISTANCE FROM TIP (IN MICRONS) OF FIRST AND LAST METAPHASES OF DIFFERENT TYPE*

ROOT NO.	LENGTH (MM.)	FIRST				LAST			
		$2n$	**$4n$**	$4n$	**$8n$**	$2n$	**$4n$**	$4n$	**$8n$**
22	4–5	.....	.....	.....	.....	.....	.....	.....	.....
23	4–5	300	.....	.....	.....	410	.....	.....	.....
25	4–5	370	.....	.....	.....	460	.....	.....	.....
20	4–5	260	440	560	.....	600	440	560	.....
2	13	250	440	550	510	470	710	740	590
16	15	170	250	.....	380	480	420	.....	380
15	15	360	520	600	590	810	720	660	750
5	25	270	490	550	.....	680	590	760	.....
28	30	320	660	580	640	760	720	580	640
17	30	270	380	620	520	890	970	710	720
30	35	280	470	550	.....	1120	700	800	.....
27	41	350	.....	.....	.....	720	.....	.....	.....
26	54	270	.....	550	.....	570	.....	610	.....
29	55	340	600	490	620	990	750	820	740
31	80	300	530	570	790	860	550	810	790
32	80	300	590	630	720	720	680	720	720

* Paired metaphase condition indicated by heavy type.

that the age of the root makes a difference in the types of divisions was an important contribution, but apparently it was not applied with sufficient precision. These workers did not actually measure the roots, nor do they give even approximate lengths to their four groups. Early in the present investigation it became evident that the age of the root made a difference in the kind of divisions found, and accordingly each root was measured at the time of fixation. Tables 1 and 2 give the lengths of the roots in millimeters and the position and kind of metaphases found in each tip. A study of embryos dissected from seeds before the beginning of germination showed that the radicle is already fully formed and differentiated at this stage. The data of tables 1 and 2 may be interpreted as follows: Young germinating roots under 4 mm. in length contain no divisions. Growth thus far is due solely to increase in cell size through intake of water. The first divisions begin in roots

4–5 mm. in length. Differentiation into the primary meristems, dermatogen, periblem, and plerome begins about 250μ from the tip. In time this differentiation takes place during embryo formation. Paired tetraploid metaphases are regularly found nearer the tip than the unpaired. Tetraploids are rarely found in roots 5 mm. long but become increasingly numerous in longer roots. Paired octoploids are first found in roots about 13 mm. in length 510μ from the tip. Figure 18 shows the distribution of metaphases of different types in the root. The fine line indicates the extreme distribution and the superimposed heavy line the average distribution. Paired metaphase classes are underlined.

TABLE 2

NUMBER AND PERCENTAGE OF TOTAL NUMBER OF METAPHASES OF DIFFERENT TYPE* IN PERIBLEM

Root no.	Length (mm.)	Number				Total	Percentage			
		2*n*	4*n*	4*n*	8*n*		2*n*	4*n*	4*n*	8*n*
22	4–5	.....	.....	.....	.....	.....	.....	.....	.....	.....
23	4–5	3	.....	.....	.....	3	100	.....	.....	.....
25	4–5	2	.....	.....	.....	2	100	.....	.....	.....
20	4–5	10	1	1	.....	12	84	8	8	.....
2	13	20	14	3	3	40	50	35	7.5	7.5
16	15	15	5	.....	1	21	71	24	5	.....
15	15	26	9	2	5	42	62	21	5	12
5	25	2	.....	.....	.....	2	100	.....	.....	.....
28	30	10	2	1	1	14	71	14	7.5	7.5
17	30	17	24	2	4	47	36	51	4	9
30	35	36	8	6	.....	50	72	16	12	.....
27	41	9	.....	.....	.....	9	100	.....	.....	.....
26	54	12	.....	3	.....	15	80	.....	20	.....
29	55	56	6	7	3	72	78	8	10	4
31	80	16	2	3	1	22	73	9	14	4
32	80	15	5	2	1	23	65	22	9	4

* Paired metaphase condition indicated by heavy type.

CRITERIA OF POLYPLOIDY

The preceding data were secured by counting metaphases only; no late prophases or early anaphases were included. There are other criteria of the degree of polyploidy which, though less certain than metaphase counts, are still of value. There is a general agreement between cell size and degree of polyploidy, although in any particular case this norm is not infallible, since one dimension of the cell is not readily available in sections. Nuclear size is somewhat more reliable than cell size, the nucleus being less variable in shape and not subject to vacuolization. Two other useful criteria were the number and size of nucleoli and the number of heteropycnotic satellites associated with the nucleolus. Both are applicable to cells in the resting stage. The first, nucleolar size and number, was discovered by DE MOL

(18) and has been used extensively by HEITZ (8); the second, satellite number, is new to cytological study (3).

NUCLEOLUS

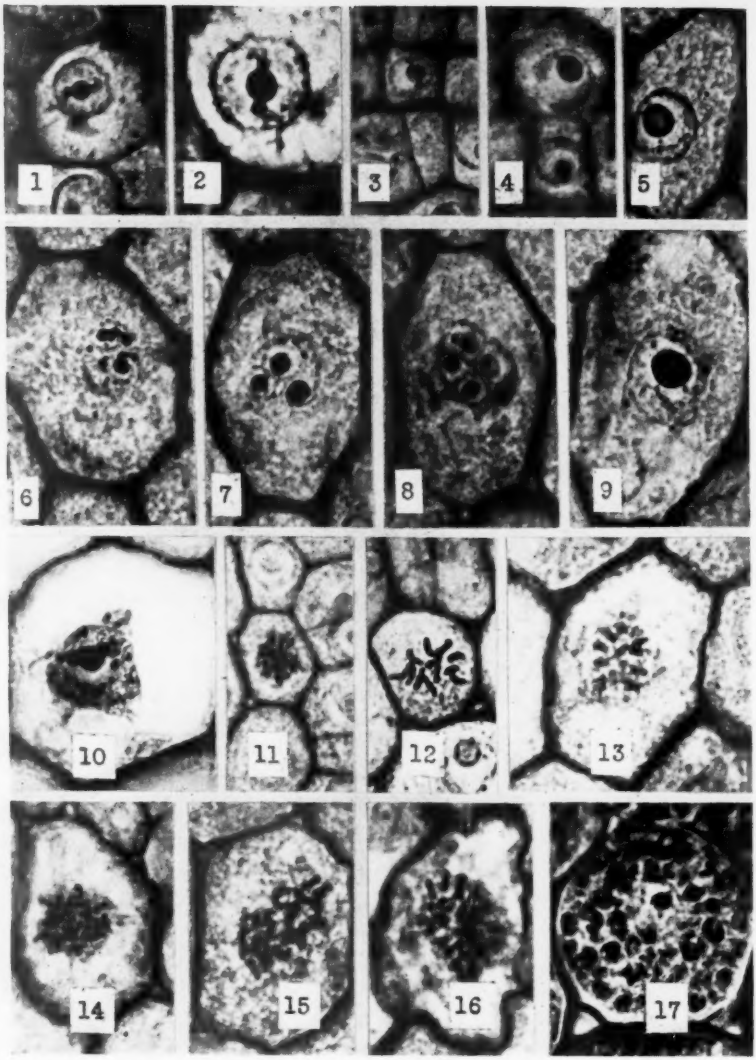
Spinacia oleracea has a diploid number of twelve chromosomes (figs. 11, 12). They have been described by LORZ (16) in great detail. One of the haploid set of six has a satellite attached by a short secondary constriction. The nucleolus is formed at this constriction. The regularity in the behavior of the nucleolus as worked out by HEITZ (8) in a number of plant species has been fully confirmed in this investigation. *Spinacia* is very favorable material for this study, not only because of the presence of several degrees of polyploidy but also because there is only one nucleolated or SAT-chromosome, and the situation does not become too complicated for analysis. Diploid cells always have either two small or one large nucleolus. In the region of the periblem where tetraploid cells are found, resting nuclei have four small nucleoli, one large nucleolus, or an intermediate number, in the process of fusion. The situation in the large cells of the octoploid region is similar, the nuclei containing a varying number of nucleoli ranging from eight small to one very large one. At telophase there is usually a number of small nucleoli which come into contact and fuse during the transition to resting stage. Transitional or early resting stages of this type have been interpreted by some earlier investigators as evidence for the origin of polysomaty by nuclear fusion. Figures 6–9 show transitional stages from telophase to resting stage of octoploid cells.

SATELLITES IN POLYPLOID CELLS

The satellite of *Spinacia* is heteropycnotic in the resting nucleus. It is either in contact with the nucleolus (figs. 1–4) or is situated near the nucleolus and joined to it by a thin thread which may be single or double (figs. 4, 5, 9, 17). The nucleolus is surrounded by a clear region. Whether this region is a fixation artifact or not is immaterial; it appears after all ordinary fixatives and is most useful in affording an unobstructed view of the satellite and its attachment thread. Diploid cells (figs. 1–4) have two satellites, tetraploid cells (fig. 5) have four, and octoploid cells (fig. 9) have eight. Older vacuolated cells which have either ceased germination divisions or have not divided since embryo formation frequently have their satellites grouped together in masses (fig. 10), from which at times threads stretch out to join the chromonemal network under the nuclear membrane. In favorable cases a count of the number of satellites or of the attachment threads is a reliable index of the degree of polyploidy of a resting nucleus.

ORIGIN OF POLYSOMATY

The various hypotheses proposed to account for the origin of the polyploid condition may be grouped into three classes: (1) Those explanations involving some



FIGS. 1–17.—Cells from various tissues of root tip of *Spinacia oleracea*. Distance of cell from tip given in each case. Fig. 1, diploid cell from calyptra showing nucleolus with two satellites (230 μ). Fig. 2, same cell at higher magnification to show double chromonemata attached to each satellite (230 μ). Fig. 3, diploid cell from calyptra; nucleolus faintly stained; satellites pycnotic (360 μ). Fig. 4, diploid cell from periblem; nucleolus with satellites not in immediate contact (360 μ). Fig. 5, tetraploid cell from periblem; one large nucleolus with four satellites, two of which show double structure (700 μ). Fig. 6, octoploid cell from periblem with many nucleoli fusing; very early resting stage after 8*n* division (700 μ). Fig. 7, same with three nucleoli, one showing two satellites; early resting stage (700 μ). Fig. 8, same with four nucleoli; early resting stage (660 μ). Fig. 9, same showing single large nucleolus with eight satellites; fully formed resting stage (700 μ). Fig. 10, same in region of elongation; cell has large central vacuole and has probably not undergone any divisions since embryo formation; satellites seen in two large groups, from one of which are many attachment threads (1040 μ). Fig. 11, diploid metaphase; outer layer of periblem (290 μ). Fig. 12, same (410 μ). Fig. 13, paired tetraploid metaphase from periblem (570 μ). Fig. 14, unpaired (740 μ). Fig. 15, paired octoploid metaphase from periblem (720 μ). Fig. 16, same (380 μ). Fig. 17, polyploid cell from periblem of radicle dissected from seed before germination; cell plasmolyzed and full of starch granules; nucleus shows evidence of polyploidy (390 μ).

kind of cell or nuclear fusion, either in normal or in binucleate cells or following some process of pseudo-amitosis or restitution nucleus formation. Theories of this type were proposed by STOMPS (**20**), BRESLAWETZ (**4, 5**), MEURMAN (**17**), HUSKINS and SMITH (**9**), and WULFF (**22**). Because of later evidence this solution must be abandoned. (2) Those solutions involving a double prophase split. This was suggested in slightly different form by DE LITARDIÈRE (**14**), LANGLET (**13**), and LORZ (**16**). In the light of recent findings on internal chromosome reproduction and of later work on polysomaty in *Spinacia* this solution must also be rejected. (3) The most convincing interpretation of the origin of polysomaty is that it arises as a result of double chromosome reproduction in the resting nucleus. The credit for this solution must be given to GENTCHEFF and GUSTAFSSON (**7**), although, owing to the results of some X-ray work, they have compromised and place the actual reproduction at some ill-defined period between resting stage and the earliest visible prophase. These workers reject the double prophase split because in favorable cases the polyploid condition, both paired and unpaired, can be seen in earliest prophase. They also note that the paired chromosomes are relationally coiled from earliest prophase, which can mean only that they have come out of resting stage in the paired condition. On both of these points the observations here recorded are in full agreement. The paired, relationally coiled, prophase chromosomes are clear and unmistakable. At late prophase, when the pairs are contracted, they take a form described by earlier workers as somatic-diakinesis. The significance of the somatic-diakinesis figures is now clear. They are late prophase stages of paired polyploid cells. The chromosomes are considerably contracted and are held together in pairs by the remaining relational coiling, which in this way simulates chiasmata.

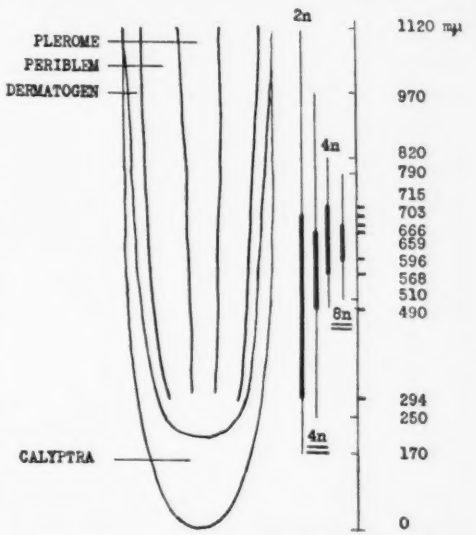


FIG. 18.—Distribution of different degrees of polyploids in root tip of *Spinacia oleracea*. Fine lines represent extreme distribution; heavy superimposed lines, average distribution. Classes twice underlined represent polyploids with paired chromosomes.

After subjecting dry seeds to X-radiation, GENTCHEFF and GUSTAFSSON (**7**) obtained in the germinating root paired and unpaired chromosome fragments but

no chromatid fragments. From this they concluded that the chromosomes in the resting nuclei of dry seeds are single. These results also led to their view that the actual doubling occurs at a stage between resting and earliest visible prophase. Without questioning the value of X-ray work in general, it is doubtful whether the singleness of the chromosomes was demonstrated. The cells of the dry seed prior to germination are highly dehydrated, the cytoplasm is plasmolyzed and shrunken away from the cell wall, the nucleus is likewise considerably shrunken and irregular in outline, being indented on all sides by encroaching starch grains. In such a nucleus the chromosomes also are no doubt dehydrated, and it is quite possible that the members of a reduplicated chromosome would be so close together that they would act as a single unit. At any rate until more is known about the condition of the chromosomes in the abnormal nuclei of dry seeds, the evidence for their singleness will remain inconclusive. Since the doubling has taken place before prophase, and since chromosome reproduction leading to polyploidy has been shown to take place during the resting stage in a number of insects, there seems no reasonable doubt that the polyploid condition in root tips is likewise due to a reduplication of the chromosomes in the resting nucleus while the nucleus itself is increasing in size and volume. In a recent paper KOSTOFF (10) mentions the polysomaty situation in passing and accepts the preceding explanation of its origin.

GENTCHEFF and GUSTAFSSON explain the apparent presence of unpaired before paired tetraploids by supposing that polysomaty may be established before germination begins. As already noted, it is doubtful whether the unpaired condition does actually precede the paired; nevertheless there is other evidence to show that polysomaty is established before germination. This evidence comes from applying some of the criteria of polyploidy just mentioned to the periblem cells of the radicle before germination and to the cells of the non-meristematic region of young germinating roots.

The seed of *Spinacia* contains a fully formed embryo. With the exception of vacuolization, all the differentiation present in young germinating roots is already present in the seed. Calyptra, dermatogen, periblem, and plerome are differentiated, and the cells of the periblem show the progressive increase in size characteristic of growing roots. There are minor differences, of course; all the cells of the radicle are plasmolyzed, and the cytoplasm is packed with starch grains. These granules stain deeply and obscure most of the nuclear details, yet in favorable cases the nuclei of the larger cells may be seen to have a polyploid number of satellites and attachment threads. Figure 17 shows a cell which is undoubtedly a polyploid (tetraploid or octoploid) by all the criteria which can be applied to a resting cell.

In connection with the origin of the polyploid condition, METZ (personal communication) raised the interesting question as to whether cells showing the higher

degrees of polyploidy had arrived at that condition by successive reproductions during a prolonged resting stage or after passing through at least one division in each of the lower degrees of polyploidy. Previous workers have not been clear on this point, but all appear to favor the latter interpretation; indeed none seems to have considered the first possibility. Some evidence on this question is available. In the mosquito (**1, 2**) high degrees of polyploidy are reached during a prolonged resting stage, and when division ensues the prophase chromosomes appear in closely associated groups of eight or sixteen sister chromatids. The condition of the satellites and attachment threads of such a cell as shown in figure 10 seems to favor a similar interpretation. This cell most probably became octoploid during embryo formation and, owing to its distal position in the radicle, did not undergo division at germination but immediately began differentiation by elongation and vacuole formation. Its multiple attachment threads and satellites are in two groups. It is possible that the polyploidy established before germination may have arisen by successive doublings of the chromosomes without the intervention of any mitosis. A thorough study of the embryology of *Spinacia* would be necessary to determine this point.

There is evidence, however, that the polyploid condition also arises after the beginning of germination during growth of the root. The paired condition and relational coiling of the chromosomes at prophase and metaphase indicate that the double chromosome reproduction has taken place during the preceding resting stage. If, therefore, the paired condition and the relational coiling are found in cells which are known to have undergone at least one mitosis since the onset of germination, this would constitute proof that the doubling had taken place since the beginning of germination. Since mitosis is confined to the first millimeter of the root tip in *Spinacia*, it seems certain that in a root several inches long all the cells of the growing point will have undergone some divisions since the beginning of germination. Roots nos. 31 and 32 in tables 1 and 2 satisfy this condition. Both were 80 mm. long when their tips were fixed, and both have many polyploid prophases and metaphases showing pairing and relational coiling.

Whatever may be the situation in polyploidy established before germination, it appears certain that polyploidy established after germination originates by a process of successive chromosome doubling, with at least one mitosis intervening at each new degree of polyploidy. This is shown by the fact that in prophases and metaphases of paired tetraploids and octoploids groups of four or eight chromosomes are never found; only pairs of sister chromosomes are observed. In an octoploid where four pairs of satellited chromosomes are present, the pairs are scattered at random. This condition is in striking contrast with that found in *Culex* and other mosquitoes, where the first division after the reduplication period shows close association of high multiples of sister chromosomes.

Summary

1. Polysomaty in the root tip of *Spinacia* is apparently restricted to the periblem.

2. The different types of polyploid cells appear in the following sequence in regard to time of appearance and position: diploid, tetraploid with paired chromosomes, tetraploid unpaired, octoploid paired.

3. Polyploidy arises by successive reproduction of chromosomes during the resting stage of the nuclear cycle.

4. The paired condition of the chromosomes indicates that the double chromosome reproduction has taken place during the resting stage immediately preceding.

5. Polyploid cells with unpaired chromosomes have undergone at least one mitotic division since the time of double reproduction.

6. The degree of polyploidy of cells in the resting stage can be judged by the number and size of nucleoli and the number of heterochromatic satellites associated with the nucleolus.

7. Polysomaty is already established in the periblem of the radicle before the beginning of germination. Double chromosome reproduction therefore takes place during embryology. Double chromosome reproduction also takes place during growth of the root after germination has begun. This is shown by pairing and relational coiling in polyploid prophases of older roots.

8. Polyploidy established after the beginning of germination is due to successive chromosome doubling, with at least one mitosis intervening at each new degree of polyploidy.

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LITERATURE CITED

1. BERGER, C. A., Multiplication and reduction of somatic chromosome groups as a regular developmental process in the mosquito, *Culex pipiens*. Publ. no. 496, Carnegie Inst. Washington. 1938.
2. ———, Prophase chromosome behavior in the division of cells with multiple chromosome complexes. Jour. Heredity **29**:351–357. 1938.
3. ———, Some criteria for judging the degree of polyploidy of cells in the resting stage. Amer. Nat. **75**:93–96. 1941.
4. BRESLAWETZ, L., Polyploide Mitosen bei *Cannabis sativa* L. Ber. Deutsch. Bot. Ges. **44**: 498–502. 1926.
5. ———, Polyploide Mitosen bei *Cannabis sativa* L. II. Planta **17**:644–649. 1932.
6. ERVIN, C. D., Polysomaty in *Cucumis Melo*. Proc. Nat. Acad. Sci. **25**:335–338. 1939.
7. GENTCHEFF, G., and GUSTAFSSON, A., The double chromosome reproduction in *Spinacia* and its causes. I; II. Hereditas **25**:349–386. 1939.

8. HEITZ, E., Die Ursache der gesetzmässigen Zahl, Form und Grösse pflanzlicher Nukleolen. Planta **12**:774–843. 1931.
9. HUSKINS, C. L., and SMITH, S. G., A cytological study of the genus *Sorghum*. Jour. Genetics **25**:241–249. 1932.
10. KOSTOFF, D., Studies on atypical growth in plants from a cytogenetic point of view. Jour. Genetics **39**:469–484. 1940.
11. KOZHUKHOW, Z. A., Karyotypische Eigentümlichkeiten der kultivierten Cucurbitaceae. Bull. Appl. Bot. Pl. Breeding **14**:89–96. 1925.
12. ———, Über experimentelle Chromosomenzahlverdoppelung in den somatischen Zellen mit abnormen Temperaturen. Angew. Bot. **10**:140–148. 1928.
13. LANGLET, O., Zur Kentniss der polysomatischen Zellkerne in Wurzelmeristem. Svensk. Bot. Tidskr. **21**:169–184. 1927.
14. LITARDIÈRE, R. DE, Les anomalies de la caryocinèse somatique chez le *Spinacia oleracea*. Rev. Gén. Bot. **35**:369–381. 1923.
15. ———, Sur l'existence de figures didiploides dans le meristeme radiculaire du *Cannabis sativa* L. La Cellule **35**:21–25. 1925.
16. LORZ, A., Cytological investigations on five chenopodiaceous genera with special emphasis on chromosome morphology and somatic doubling in *Spinacia*. Cytologia **8**:241–276. 1937.
17. MEURMAN, O., Chromosome morphology, somatic doubling and secondary association in *Acer platanoides* L. Hereditas **18**:145–173. 1933.
18. MOL, W. DE, Nucleolar number and size in diploid, triploid and aneuploid hyacinths. La Cellule **38**:7–65. 1927.
19. RANDOLF, L. F., A new fixing fluid and a revised schedule for the paraffin method in plant cytology. Stain Technol. **10**:95–96. 1935.
20. STOMPS, T. J., Kernteilung und Synapsis bei *Spinacia oleracea*. Biol. Centralbl. **31**:267–309. 1910.
21. TUSCHNJAKOWA, M., Untersuchungen über die Kernbeschaffenheit einiger diözischer Pflanzen. Planta **7**:29–44. 1929.
22. WULFF, H. D., Die Polysomatie der Chenopodiaceen. Planta **26**:275–290. 1936.

RESPONSES OF VEGETATIVE PARTS OF PLANTS FOLLOWING APPLICATION OF EXTRACT OF POLLEN FROM ZEA MAYS

JOHN W. MITCHELL[1] AND MURIEL R. WHITEHEAD[2]

(WITH TWELVE FIGURES)

Introduction

The existence of growth-stimulating properties in reproductive bodies of some plants was first observed in connection with the development of fruits. As early as 1849 it was found that application of spores of *Lycopodium* to the stigmas of certain cucurbits resulted in growth and development of seedless fruits (**4**). Parthenocarpic development of fruit has been induced also by applying pollen of other species to the stigmas of certain cucurbits, grapes, and orchids (**14, 15**), whether such pollen were dead or living. More recently, extracts of pollen capable of inducing parthenocarpy have been obtained, thus indicating that the stimulus to develop is chemical in nature (**3, 7, 12, 13, 23**). LAIBACH and KORNMANN (**12**) applied extracts of orchid pollen to plant parts other than the carpels; they observed that when an extract of pollen was applied to them unilaterally, oat coleoptiles bent and elongated more rapidly when bathed in a solution of pollen extract. They also observed that epicotyls of *Phaseolus* and petioles of *Coleus* bent following unilateral applications of pollen extract. LA RUE (**13**) demonstrated that extracts of pollen retarded the formation of abscission layers in debladed petioles of *Coleus*. Similar responses have also been effected by applying various synthetic growth-regulating substances to the stems and fruits of some plants (**1, 2, 5, 6, 19, 20, 22**).

The investigations here reported deal with studies of growth responses and histological changes resulting from application of extracts of corn (*Zea mays*) pollen to different vegetative parts of plants. A detailed study was made of responses that occurred when the extract was applied to the cut surface of decapitated stems, or to the epidermis of the stems of intact plants, and these were compared with those that resulted when certain synthetic growth-regulating substances were applied in a similar manner.

Methods

The pollen used in these experiments was collected mainly from a single cross of two varieties of yellow field corn (KYS × US7), in July, 1940, although similar work was done in the autumn of 1939 also. Tassels having anthers about to de-

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hisce were covered with paper bags and the pollen collected as it was shed. Anthers mixed with the pollen during its collection were separated by screening and discarded. The pollen was dried at 80° C. in a well-ventilated oven and stored in airtight glass containers, this method proving suitable, as illustrated by the fact that material thus stored for about one year still yields quantities of active extract.

Extractions were made by adding ether to pollen in proportions of 20 ml. to 5 gm. of pollen and allowing the mixture to stand over night at room temperature. The mixture was then filtered and the filtrate collected in a weighed evaporating dish. The ether was finally evaporated over a water bath at 50–60° C. until the residue attained a constant weight. A fatty residue weighing 25–35 mg., and having a melting point of 46–48° C., was obtained in this way.

With the exception of a few experiments, Baker's analyzed ethyl ether was used as a solvent with which the growth-regulating substance was extracted from the pollen (the number of such substances extracted is not known, but for simplicity the active fraction of the extracts is referred to as growth-regulating substance). The ether was not purified as suggested by VAN OVERBEEK (**21**), since no difficulty was experienced in obtaining highly active extracts without taking this precaution.

For use in experimental work, anhydrous lanolin was thoroughly mixed with the fatty residue remaining after evaporation of the ether, generally in proportions of one part by weight of extract to four parts of lanolin. This mixture was then applied by gently rubbing a small amount carefully on the surface of a plant so as to insure contact but to avoid rupturing of cells or cuticular layers.

For part of the histological studies, approximately 10–15 mg. of the paste was applied to the cut surfaces of decapitated second internodes in the same manner as already described in connection with similar studies (**9**). In other experiments it was applied to the surface of intact first internodes of non-decapitated bean plants, and to some other plants, as detailed later. In the case of bean, young vigorous seedlings were used, the applications being made when the first internode was approximately 15 mm. long, the heart-shaped leaves not fully developed, nor the second internode elongated. The stems were preserved in Navashin's solution, then sectioned with the aid of a freezing microtome, or imbedded in paraffin and sectioned in the usual manner.

In determining the size of cells it was not feasible to measure those of tissues made up of relatively few or small cells. The measurements as recorded are based on parenchymatous cells of the cortex and pith. The average number and size of such cells was determined by first selecting internodes of average length. From each of these internodes, segments 2 mm. long were removed at 5-mm. intervals and sectioned longitudinally. In one median section taken at each 5-mm. level an approximate row of cells extending from one side of the section to the other

was selected for measurement. Thus it was possible to compute the average width and length of cells of these tissues at regular intervals along the internode and to determine the dimensions of average cells for a given tissue.

EXTRACTION AND SOLVENTS.—The solubility of the active substance was tested by adding 40 ml. of ethyl ether, chloroform, acetone, or absolute alcohol, to 10-gm. lots of pollen and allowing the mixtures to stand at room temperature over night. The filtrates, with the exception of the aqueous ones, were evaporated to dryness on a water bath. The waxy residues were mixed with four times their weight of lanolin, and finally 50–75-mg. aliquots of these mixtures were applied unilaterally to the stems of bean seedlings. Filtrates of water extracts were evaporated at reduced pressure at 60° C. to a volume of 1–2 ml., and the liquid then mixed with 1 gm. of lanolin. As in the case of the other residues, 50–75-mg. aliquots were applied unilaterally to stems of young bean plants. All four organic solvents gave active extracts. Extracts with hot or cold water did not contain an appreciable amount of growth-promoting substances in an active form. Ether extracts of pollen which had previously been extracted with water and dried also failed to give positive tests, indicating the possibility that the growth-stimulating substance may have been soluble but unstable in water. As indicated by increased linear growth of bean stems, ether was most effective in extracting the growth-regulating material, and this solvent was therefore used in all subsequent experiments.

A number of different kinds of oils were tested as possible media in which the active substance extracted from pollen might be applied to plants. Sixty mg. each of cod liver, soybean, olive, and peanut oils were added respectively to 20 mg. of ether extract and applied unilaterally to the hypocotyls of young bean seedlings. Marked stem curvatures were observed in all cases, while plants treated with the various oils alone showed no response.

Gross responses

COMPARISON OF POLLEN EXTRACT AND SYNTHETIC GROWTH-REGULATING SUBSTANCES

The effectiveness of relatively high concentrations of various synthetic growth-regulating substances in stimulating stem elongation was compared with that of the natural growth-regulating substance extracted from pollen. Two per cent lanolin mixtures of various growth substances were applied as a band approximately 2 mm. wide around the stem midway between the first and second nodes of young bean seedlings.

One week following treatment, when elongation of the first internode had practically ceased, those internodes to which pollen extract had been applied were significantly longer than controls, by approximately 43 per cent (table 1; fig. 1).

Earlier during the experiment internodes treated with pollen extract were more than twice the length of comparable controls, but subsequent growth by the controls resulted in a relative decrease of this difference when the internodes had ceased to elongate. There was a significant increase in rate of elongation during the first few days following treatment with indoleacetic acid, but subsequently the internodes to which this substance was applied ceased to elongate, formed tumors, and were at maturity 20 per cent shorter than controls.

Phenylacetic acid, naphthaleneacetic acid, and naphthalene acetamide mixtures resulted in significant retardation of stem elongation, this response being associ-

TABLE 1

EFFECT OF COMPOUNDS ON INTERNODAL ELONGATION WHEN APPLIED AS BAND 2 MM. WIDE AROUND MIDDLE OF FIRST INTERNODES OF YOUNG KIDNEY BEAN PLANTS. FIGURES REPRESENT AVERAGE LENGTH IN MILLIMETERS OF FIRST INTERNODES OF TWENTY PLANTS

Hours after treatment	Treatment					
	Control	Pollen extract	Indole-acetic acid	Phenyl-acetic acid	Naphtha-leneacetic acid	Naphtha-lene acetamide
52	12.9	26.2*	15.6*	13.3	13.2	13.4
100	25.9	39.8*	20.6†	20.7†	17.6†	21.6†
172	28.5	40.8*	22.7†	23.2†	17.9†	24.4†

* Significantly longer than control of same date; ratio 19:1.
† Significantly shorter than control of same date; ratio 19:1.

ated with tissue proliferation and root formation in the case of the acids (**8**) and lignification and increased cambial activity in the case of the amide (**10**).

It is known that the application of small amounts of indoleacetic acid may result in cell elongation, as indicated by curvatures induced in *Avena* coleoptiles and the stems of succulent plants (**2, 22**). A comparison was made between the amount of stem elongation which resulted from the application of small amounts of indoleacetic acid and that resulting from the use of pollen extract. A 0.002 per cent lanolin mixture of indoleacetic acid was used, as previous experiments had shown that this concentration caused the greatest amount of stem elongation of any concentration tested between 0.00002 and 2.0 per cent. Internodes treated with either pollen extract or the acid were significantly longer than comparable controls treated with lanolin alone, even as early as 24 hours after treatment. Although both the extract and the acid appreciably stimulated stem elongation, the responses were unlike in two ways. First, indoleacetic acid stimulated growth in length of stems for a relatively short time, while those treated with pollen extract continued to elongate for a longer period following treatment. Second, growth

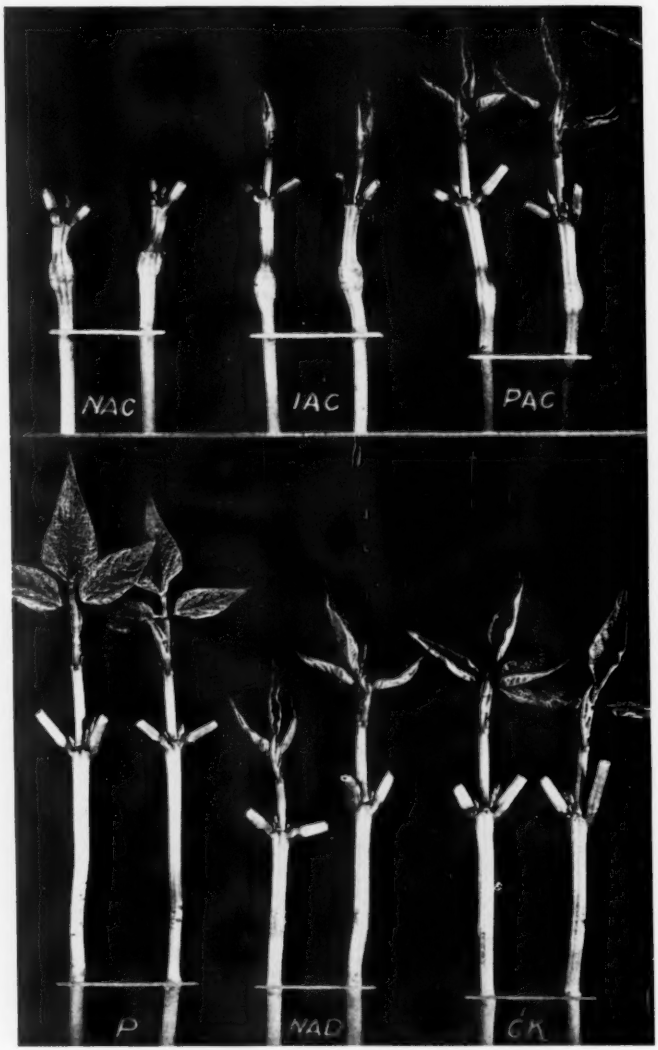

FIG. 1.—Growth responses resulting from application of 2 per cent lanolin mixtures of growth-regulating substances to first internodes of bean plants, as compared with that resulting from use of pollen extract or lanolin alone. Top: alpha naphthaleneacetic acid, beta indole-3-acetic acid, phenylacetic acid. Below: pollen extract, alpha naphthalene acetamide, and check.

stimulation as the result of application of the extract was approximately 2.6 times the maximum amount resulting from application of indoleacetic acid (fig. 2).

Still further tests were carried out to determine the effectiveness of pollen extract in stimulating linear growth. Only slight curvatures resulted when pollen extracts were applied to oat coleoptiles. Lanolin containing pollen extract in the ratio of 4:1 was applied unilaterally at one point on the upper third of the coleoptile. Somewhat stronger curvatures resulted when the extract was smeared along one side of the coleoptile for 1 cm. or more.

The application of pollen extract to the stems of different varieties of plants accelerated their rate of elongation. Plants tested included radish, flax, potato, kidney bean, soybean, marigold, tomato, *Mirabilis*, and buckwheat.

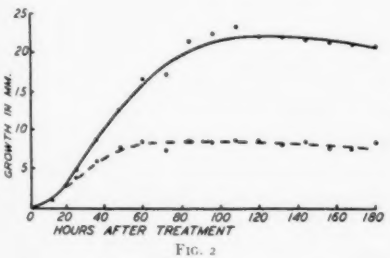

FIG. 2

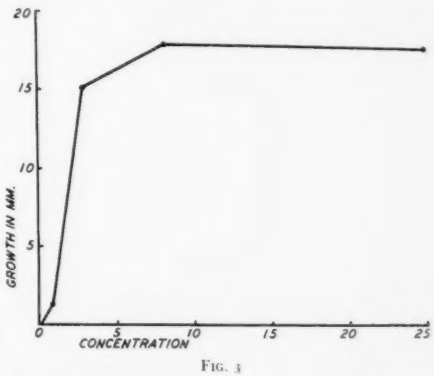

FIG. 3

FIGS. 2, 3.—Fig. 2, growth of first internodes of bean plants treated with pollen extract-lanolin mixture, compared with that of others treated with 0.002 per cent mixture of indoleacetic acid in lanolin (broken line). Values represent gain over that of controls treated with lanolin. Fig. 3, linear growth of first internodes of bean plants following treatment with different amounts of pollen extract. Values represent gain in length over that of controls during 48-hour period following treatment.

EFFECTS OF VARIOUS AMOUNTS OF POLLEN EXTRACT ON LINEAR GROWTH

To establish a quantitative relationship between the amount of extract applied to stems and their rate of elongation, bean plants were grown from seeds under controlled environmental conditions (**17**). The temperature of the room was 73–75° F., the relative humidity 65–85 per cent. Illumination was supplied for a period of 14 hours daily by means of a 60-ampere carbon arc burning Sunshine carbons and a number of 200-watt incandescent lamps evenly distributed approximately 3 feet above the plants, which together supplied an intensity of 1200–1400 foot candles.

Four concentrations of extract were made by first adding 800 mg. of lanolin

to 200 mg. of extract. This mixture was designated a 25 per cent concentration. Subsequent dilutions were made by adding 240, 960, and 3120 mg. of lanolin to 150-mg. aliquots, respectively, of the 25 per cent mixture. When the first internodes of a number of selected seedlings were approximately 15 mm. long, the plants were divided into five groups, each containing twenty-four plants. Each group was then treated by carefully applying 15 mg. of one of the four concentrations in a thin layer extending around the stem as a band 1 cm. wide. The fifth group was treated with lanolin alone as the control.

The effect of these various amounts of extract on the rate of internodal elongation was measured at intervals during the 3 days following treatment. Maximum stimulation resulted when the mixture contained 10 per cent of crude extract (fig. 3). Further dilution resulted in a marked decrease in the amount of internodal elongation. It was concluded that bean seedlings, grown under these conditions, could be used as test subjects for quantitatively measuring different amounts of substances extracted from corn pollen, which stimulate linear growth.

Mobilization effects of pollen extract

It was previously found that stems of kidney bean formed tumors as a result of tissue proliferation following applications of 2 per cent lanolin mixtures of indoleacetic or naphthaleneacetic acids (**18, 19**), and that this response was associated with the rapid movement of some organic substances toward the treated region. As a result of this mobilization of materials, treated portions of stems gained in dry weight more rapidly than did comparable portions of untreated stems.

It was observed that mobilization was also associated with an increased rate of stem elongation as induced by application of the pollen extract. To demonstrate this effect, a band of pollen extract-lanolin mixture was applied to the first internodes of Biloxi soybean seedlings when the internodes were approximately 15 mm. long. After the plants had been grown for 18 days in a greenhouse, the treated internodes averaged approximately 53 per cent longer and 54 per cent heavier in fresh weight than did comparable parts of controls treated with lanolin alone. Growth of the stem and leaves above the treated internode was significantly retarded, the area of the primary leaves being approximately 22 per cent less than that of controls at the end of the experiment.

In a subsequent experiment the effect of pollen extract on the accumulation of dry weight in the stems of kidney bean plants was determined. The plants were treated by placing a band of the pollen extract-lanolin mixture around the first internode when these were approximately 15 mm. long. Three days following treatment, the internodes to which the extract had been applied had gained 65 per cent more in length, 86 per cent more in fresh weight, and 81 per cent more in dry weight than had the first internodes of comparable controls treated with lanolin

alone. It was concluded that stem elongation resulting from application of the pollen extract was associated with movement of materials toward the treated region.

Effects of light on elongation

It is known that in certain plants internodal elongation is limited to some extent by the quality and intensity of light with which they are illuminated (**16**). A number of experiments were carried out to determine the effect of light intensity and quality on the response of bean internodes following the application of pollen extract.

Bean seedlings were grown under controlled environmental conditions (**17**). Some of the plants were supplied with a light intensity of 200 foot candles, others

TABLE 2

Effect of light intensity on stem elongation following treatment with pollen extract-lanolin mixture. Figures represent average length in millimeters of twelve plants

Hours after treatment	Treatment					
	1800 foot candles			200 foot candles		
	Extract mixture	Lanolin	Difference	Extract mixture	Lanolin	Difference
48	48.2*	29.7	18.5†	48.8*	39.8	9.0
72	56.4*	38.8	17.6†	59.7*	49.4	10.3
96	59.8*	45.0	14.8†	63.6*	54.0	9.6

* Significantly longer than control of same date grown under same light intensity; 19:1.
† Significantly greater than difference at intensity of 200 foot candles; 19:1.

with an intensity of 1800 foot candles, of light from a carbon arc. Greatest increase in linear growth over that of controls resulted from application of the pollen extract to the internodes of plants grown at the higher intensity (table 2). In other experiments even greater increase resulted when treated plants were grown in natural daylight of a relatively high intensity, while little or no increase over controls was observed when the plants were grown in complete darkness.

The quality of light was varied, under controlled conditions, by illuminating a group of plants with light from incandescent lamps which supplied an intensity of 700 foot candles at the leaf surface, while another group was illuminated with approximately the same intensity of light supplied by means of Daylight fluorescent tubes. Plants grown under the fluorescent tubes produced relatively short stems, while those grown under the incandescent lamps developed elongated internodes. Internodal elongation resulted following the application of pollen extract only in the case of those plants illuminated by means of fluorescent tubes (table 3).

In summary, it was observed that the greatest increase in linear growth resulted when the extract was applied to internodes of plants grown in light of an intensity and quality most favorable for the development of short sturdy stems, and there was little or no difference between internodal elongation of treated and control plants grown under light conditions that favored etiolation.

Effects of pollen extract on structures other than first internode

To determine the effect of pollen extract on the growth of vegetative parts of bean plants other than the first internode, buds, leaf tissue, second and third internodes, and roots were treated. Terminal vegetative buds approximately 3 mm.

TABLE 3

Effect of light quality on internodal elongation following treatment with pollen extract. Figures represent average length in millimeters of first internodes of thirty-six plants

Hours after treatment	Treatment					
	Fluorescent light			Mazda light		
	Extract mixture	Lanolin	Difference	Extract mixture	Lanolin	Difference
48	31.6*	13.9	17.7	35.3†	46.7	11.4
96	48.9*	25.3	23.6	61.2†	64.5	3.3
144	50.1*	31.5	18.6	68.5	68.7	0.2

* Significantly longer than control of same date grown in same light quality; 19:1.
† Significantly shorter than control of same date grown in same light quality; 19:1.

long, and others 1 cm. long, were coated with a thin layer of melted pollen extract-lanolin mixture. Individual measurements made at repeated intervals following treatment showed no significant increase in either the length or the fresh weight of treated as compared with untreated buds. When the mixture was applied as a 5-mm. strip across the lower surface of young bean leaves, cell enlargement was greatly stimulated near the treated region, causing the leaves to bend at this point and curl upward and backward so that the distal end rested against the petiole end. Similar treatments applied to the upper surfaces of leaves failed to cause noticeable curvatures of the blades.

Elongation of second and third internodes of kidney bean plants was increased as a result of the application of the extract-lanolin mixture.

To test the effect of the extract on elongation of roots, kidney bean seeds were germinated in sand. When their tap roots were approximately 25 mm. long the plants were carefully removed from the sand, 120 uniform plants selected, and di-

vided into six lots of twenty plants each. The tap root of each plant was measured, and then the plants in three of these groups were treated by applying a band 3 mm. wide of the extract-lanolin mixture around each tap root at the region of elongation. A comparable number of plants were treated in the same way with lanolin to which no extract was added, as controls. The seedlings were kept moist, and each group was replanted individually in sand immediately following treatment. Twenty-four hours after treatment the plants were again removed from the sand and the tap roots measured. None of the roots showed signs of injury. Small tumors, approximately twice the diameter of the root, developed immediately distal to the treated region to which the extract-lanolin mixture was applied. The average increase in the length of roots treated with the mixture was 24.6 mm., that of controls 27.3 mm., a difference statistically significant at the 5 per cent level, indicating that applications of pollen extract-lanolin resulted in the inhibition of root elongation.

Histological responses

Lateral applications

Histological examinations showed that the main effect of lateral applications of pollen extract to first internodes resulted in an increase in the length of the

TABLE 4

AVERAGE LENGTH, WIDTH, AND NUMBER OF PITH CELLS IN FIRST INTERNODES OF BEAN STEMS TREATED WITH LANOLIN COMPARED WITH LENGTH, WIDTH, AND NUMBER OF OTHERS TREATED WITH POLLEN EXTRACT-LANOLIN MIXTURE. LENGTH AND WIDTH EXPRESSED IN MICRONS (SEE METHODS)

Internode	Treatment					
	Lanolin			Extract mixture		
	No. of cells per internode	Average length of cells	Average width of cells	No. of cells per internode	Average length of cells	Average width of cells
I	824	6.5	5.8	707	14.5	5.4
II	745	7.3	6.4	778	13.7	6.1
III	963	6.3	5.8	749	13.6	5.9
IV	761	7.1	6.0	796	13.2	5.7
V	753	7.6	5.7	790	13.1	5.8

cells of various stem tissues. The average length of pith cells of internodes treated with extract was approximately 94 per cent greater than that of pith cells of comparable controls (table 4; fig. 5). The cells having the greatest average length, 246 per cent greater than that of controls, occurred in that portion of the pith directly beneath the surface to which the extract was applied (fig. 4). Pith cells proximal or distal to this treated portion were less affected; and at the ends of the

internode, approximately 1 cm. away from the treated region, their length was only slightly greater than that of controls. Measurements showed that the growth in length and width of cortical cells was stimulated by the application of extract, particularly near the treated region. No abnormal twisting or tearing of tissues could be observed which indicated that tissues other than pith and cortex elongated correspondingly. It was evident that the increase in length of internodes as the result of treatment with pollen extract was associated mainly with increase in the length of cells in the pith and cortex, rather than with cell division. This appeared to be true in other tissues, although direct measurements were not made. The increased internodal length associated with the application of a 0.002 per cent indoleacetic acid paste was also characterized by an increase in the length of the cells of various tissues.

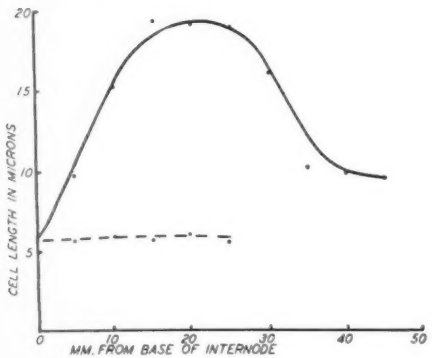

FIG. 4.—Average length of cells in pith of first internode of bean plants 96 hours after surface of internode was treated with pollen extract-lanolin mixture, compared with average length of pith cells of control treated with lanolin (broken line).

APICAL APPLICATIONS

The second internodes were decapitated just as the first trifoliate leaf was expanding, the usual care being exercised in pressing the lanolin-pollen extract mixture firmly against the cut surface. The plants were maintained under usual greenhouse conditions, and experiments were repeated at several seasons during the year. Except for slight variations in degree and extent of development of the apical tumors, the sequence of events as given here is representative.

At the end of 36 hours following treatment, the epidermal, cortical parenchyma, and endodermal cells show considerable radial elongation. For the most part the chloroplasts and starch have disappeared, the contents of the endodermal cells are more dense, and in many instances some of these have divided tangentially at least once (fig. 6). The pericyclic, phloem, and cambial cells show no apparent response.

At the end of 60 hours the epidermal cells have elongated radially still more and may have divided radially, particularly in the vicinity of an epidermal hair or a stoma. The content of the cortical parenchyma cells is very dense, and the cells have divided in all planes, the divisions being numerous just beneath the epidermis or near a stoma. The endodermal cells have undergone several divisions, both radially and tangentially, but in many instances the derivatives are not readily

distinguishable from those of the cortical parenchyma (fig. 7). In other instances the endodermal derivatives are clearly distinguishable from near-by cells, and many of them have differentiated as tracheids, especially those adjacent to the ray cells, while others continue to proliferate and form a wide band of meristematic cells

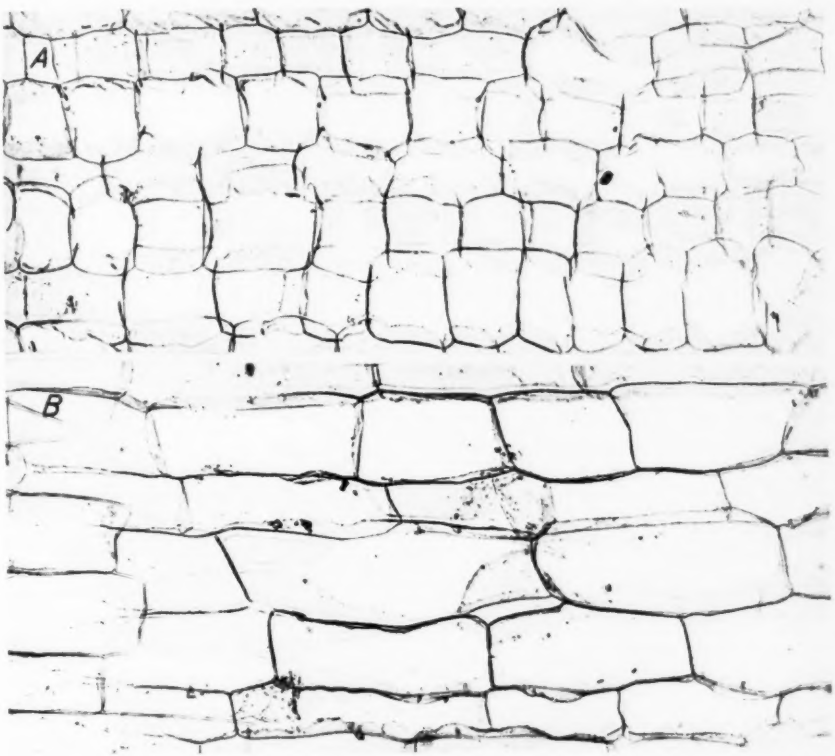

FIG. 5.—Representative pith cells of first internode of bean plants 96 hours after outer surface had been treated with ring of lanolin (*A*) compared with those of comparable internode treated in same way with pollen extract-lanolin mixture (*B*).

completely capping phloem and pericyclic cells. Some of the cortical cells are arranged as meristematic bands extending from the epidermis just beneath a stoma to the cells of the endodermis or in some instances directly to those of a ray. The ray cells themselves are but slightly meristematic, but from some of these the interfasicular cambium originates, while others divide several times and their derivatives mature as tracheids. Cells of the pith, cambium, and phloem show little or no response, other than that the former enlarge somewhat, occasionally divide,

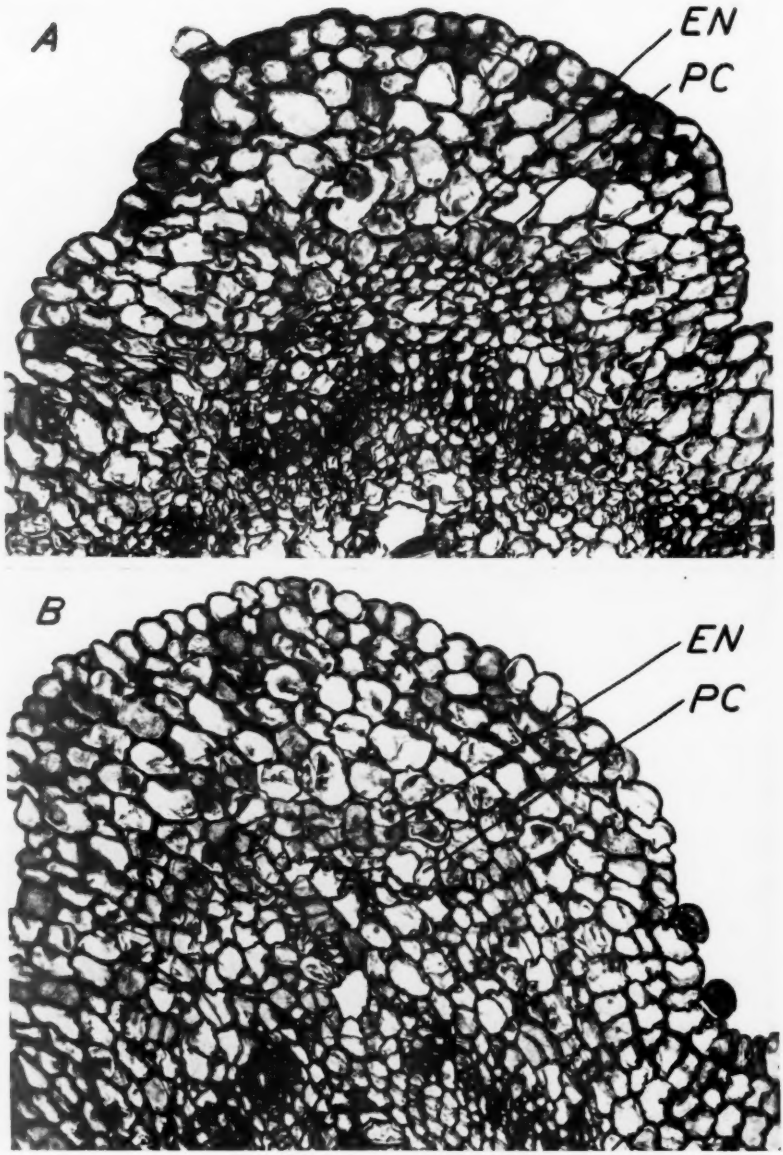

FIG. 6.—Transections of bean stem decapitated, and cut surface treated with pollen extract-lanolin mixture, 36 hours after application. Cells of epidermis, cortex, and endodermis enlarged appreciably. *A*, endodermal cells enlarged and contents more dense; *B*, many of these have divided tangentially.

and remain more active than do similar cells of plants treated with lanolin only (fig. 8).

At the end of 86 hours the same types of change, growth, and development still continue. Many more cells have matured into tracheids, however, and this is particularly true in the case of the cells derived from the endodermis. Sieve tubes and

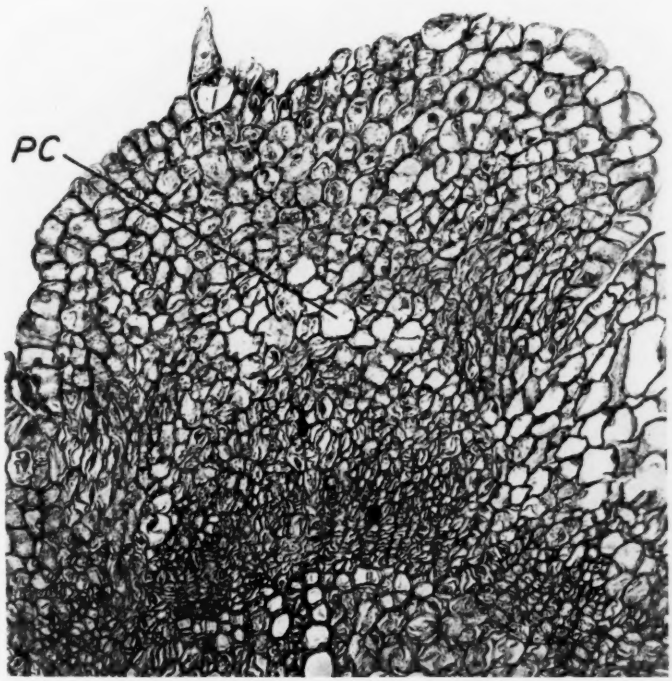

FIG. 7.—Transection near treated surface 60 hours after application. Many cortical cells have divided in various planes; pericyclic fibers have enlarged somewhat, but their walls have not increased appreciably in thickness; other tissues have remained active but show no marked meristematic activity. Proliferated endodermal cells not readily distinguishable from rest of cortex.

companion cells have also matured from derivatives of similar origin. Direct connections with the phloem and cambium of the stele made through the rays are mature and clearly delimited. The cortical parenchyma cells are active, and from the strands of meristematic cells larger numbers of scattered tracheids have matured. The vascular strands extend to a stoma. The epidermal cells continue active radial division and show no rupturing, despite the expansion of tissues beneath them (fig. 9).

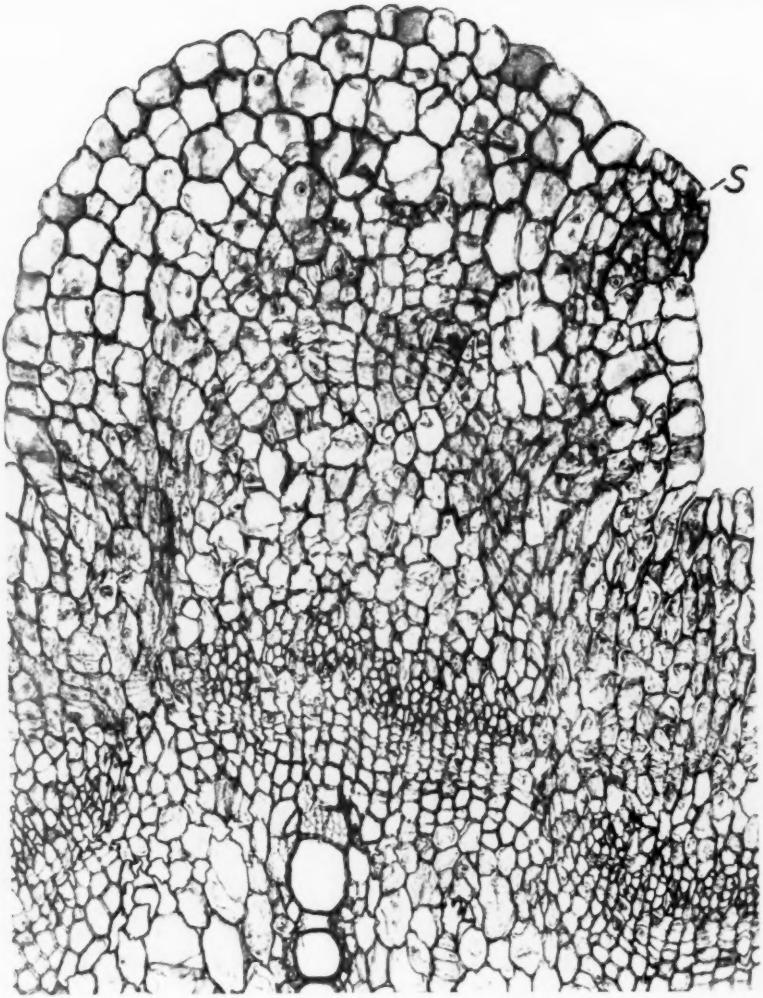

FIG. 8.—Section at about same level and at same period after treatment as fig. 7. Endodermal derivatives clearly distinguishable, some of them differentiated as tracheids near ray cells. Cortical parenchyma active, particularly the band of cells below a stoma (*S*) extending to endodermis. A few of the cells of the primary phloem have also divided.

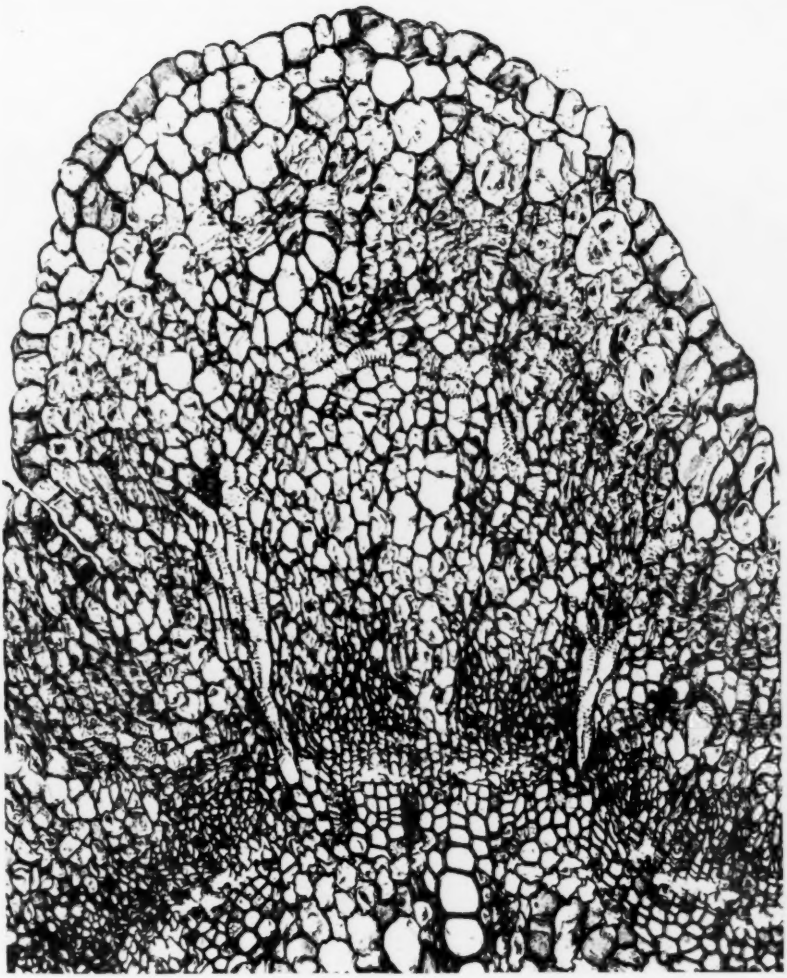

FIG. 9.—Section 86 hours after treatment. Many endodermal derivatives have matured as tracheids, especially above the rays. Most of the cortical cells have divided, some derivatives mature as tracheids, others as bands of meristematic cells which may differentiate as vascular elements. In the secondary phloem is a group of meristematic cells derived from activity of the parenchyma. These cells may differentiate and give rise to a vascular strand, or die and disintegrate as in fig. 11. Cambium has continued development, and from it has differentiated secondary tissue much as if the stem had not been decapitated.

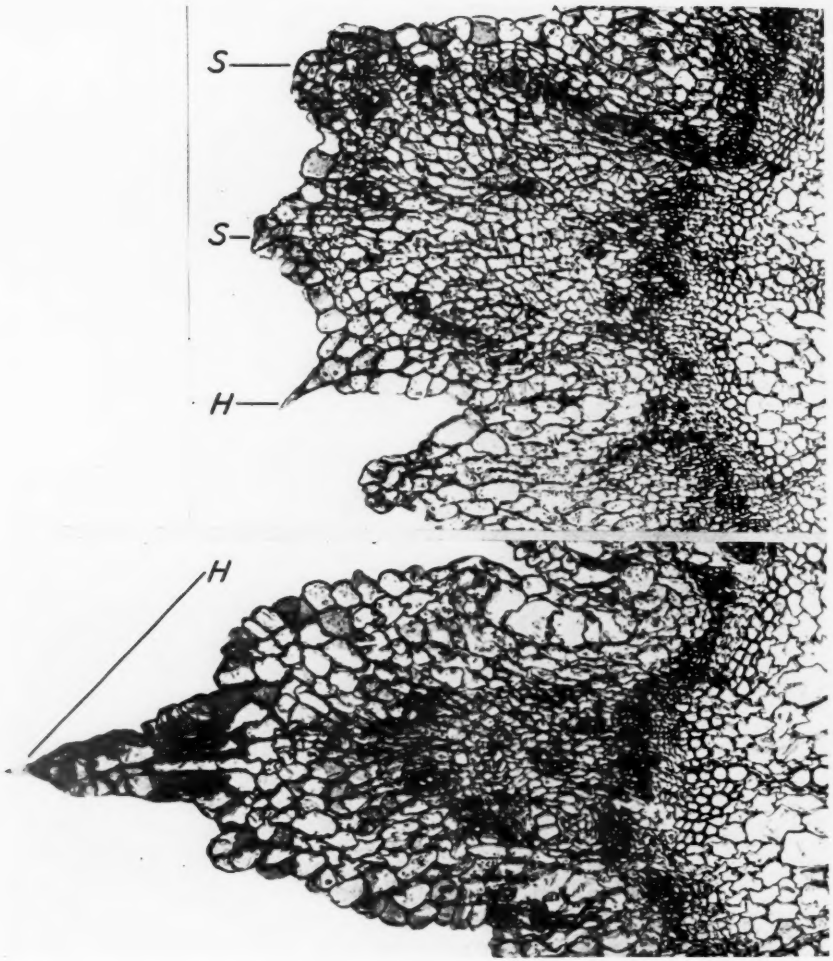

FIG. 10.—Section 186 hours after treatment. *A*, characteristic tracheids derived from endodermal and other cortical cells, and bands of vascular and provascular tissue which extend to cones of tissue associated with development of cells beneath a stoma. *B*, some of these same structures are obvious, and an epidermal hair has been pushed outward by development of a narrow cone of tissue derived from cortical parenchyma. In similar instances, which are common, the epidermal cells divide radially and enlarge so that the epidermis remains continuous and unbroken.

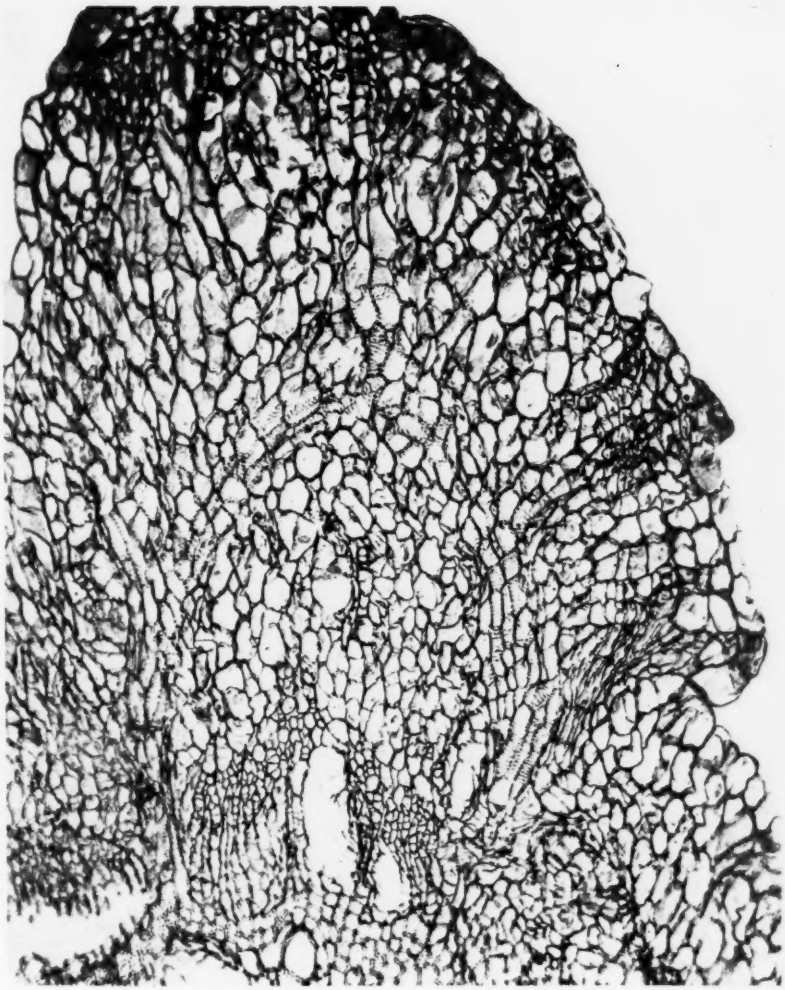

FIG. 11.—Section near treated surface 180 hours after application of pollen extract-lanolin mixture. Although many cells are still actively proliferating, particularly just beneath the epidermis, many also have matured and are no longer actively dividing. In some instances the cells have died and cracks are being formed as result of disintegration and breaking. Many tumors develop only slightly beyond this stage, although some continue to grow or at least remain alive for much longer periods.

The same type of development continues for considerable time, but 180–200 hours after treatment the apical tumors enlarge very slowly or not at all. They may remain alive for much longer periods but many of them began to die and shrivel at about that time. At 180 hours the cells of cortical parenchyma may still be active, especially just beneath the epidermis, where there are groups of meristematic cells separated by others which have enlarged and matured as thin-walled cells or as tracheids. The groups of cells are generally connected with strands of similar active cells extending to the tracheids matured from endodermal derivatives, or they may be in direct connection with the vascular elements of the stele through derivatives of the rays. As noted, these strands frequently terminate beneath a stoma, and the guard cells are pushed outward by the development and maturation of cortical tissues beneath them, until they stand at the apices of conical or variously shaped masses of tissues of considerable size, the epidermis remaining continuous owing to the continued division and enlargement of its cells. In somewhat similar manner the epidermal hairs are frequently carried outward at the ends of long points or cones of tissue, but in these cases it is uncommon for a vascular strand to be differentiated to the surface as it is below the guard cells (fig. 10). The cells of the phloem may or may not show proliferation; if at all, it is only slight; but they remain turgid and show no sign of collapse or disintegration. In a few instances scattered tracheids surrounded by sieve tubes and companion cells are differentiated in the primary phloem. Frequently rifts and cracks occur indiscriminately among the various tissues which constitute the tumor, despite the fact that actual meristematic regions are also present (fig. 11).

Compared with control plants treated with lanolin only, those to which the pollen extract has been applied show continued development of the pith, rays, cambium, secondary xylem, and phloem, which closely parallels undecapitated and untreated internodes of the same age; whereas the controls generally cease to grow, the tissues collapsing and shriveling. Occasionally such a control may continue some development, usually a proliferation of the phloem cells (fig. 12), as has already been described (9). In other cases, the cells of the endodermis, the outer portion of the rays (the cortical parenchyma particularly), and those of the epidermis enlarge greatly, become actively meristematic, and derivatives from all but the epidermis differentiate as parenchymatous tissue and vascular elements, especially as tracheids. Many of these are elongate, long pointed, and coarsely reticulate.

In general, histological responses following the application of pollen extract-lanolin mixtures to the cut surface of decapitated internodes of bean stems were unlike those recorded for indoleacetic and naphthaleneacetic acids (8), but more closely resemble those that resulted following the application of tryptophane (11).

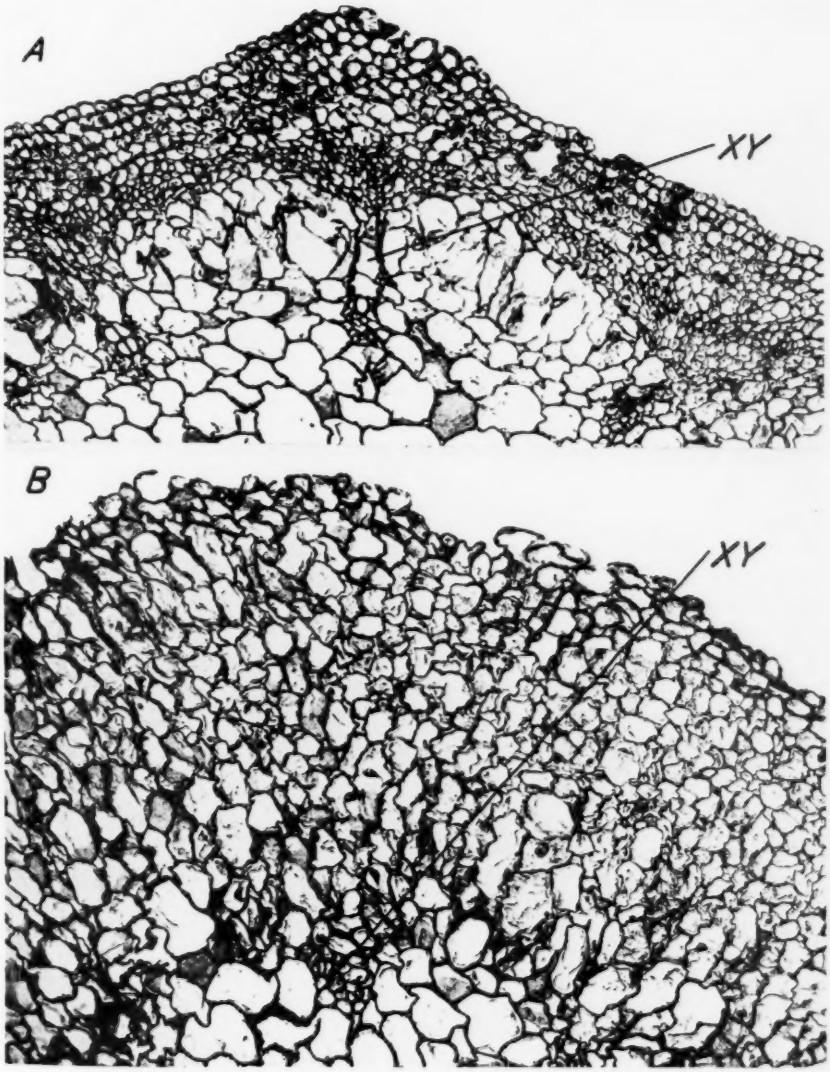

FIG. 12.—*A*, decapitated stem 86 hours after treatment with lanolin only. Although some cells were still alive there was little development after decapitation, and many cells have disintegrated. *B*, similar section immediately below treated surface 180 hours after decapitation and treatment. Phloem parenchyma proliferated to some extent. Most cells have died, although tumors of this character may continue development for considerable time.

Summary

1. Extracts from pollen of *Zea mays* mixed with lanolin were applied to certain vegetative parts of various species of plants, particularly to those of *Phaseolus*.

2. Application of the extract in the form of a ring around the stems of bean plants resulted in marked internodal elongation, which was associated mainly with an increase in the length of the cells of the various tissues, rather than with an increase in the rate of cell division. Small amounts of indoleacetic acid applied in a similar way also resulted in increased linear growth of internodes, but to a less extent.

3. Greatest increase in linear growth of bean stems resulted when the extract was applied to internodes of plants grown in light of an intensity and quality most favorable for the development of short sturdy stems, and there was little or no difference between internodal elongation of treated and control plants grown under light conditions that favored etiolation.

4. Small tumors generally resulted following application of the pollen extract to the cut surface of decapitated second internodes of bean plants. Histological responses associated with the growth of these tumors were unlike those that resulted following the application of relatively concentrated mixtures of indoleacetic or naphthaleneacetic acids and lanolin, but more closely resemble those recorded for tryptophane.

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LITERATURE CITED

1. BAUSOR, S. C., REINHART, W. L., and TICE, G. A., Histological changes in tomato stems incident to treatment with β-naphthoxy-acetic acid. Amer. Jour. Bot. **27**:769–779. 1940.
2. BOYSEN JENSEN, P. (AVERY-BURKHOLDER), Growth hormones in plants. New York. 1936.
3. FITTING, H., Die Buinflussing der Orchideenblüten durch die Bestäubung und durch andere Unstande. Zeitschr. Bot. **1**:1–86. 1909.
4. GAERTNER, K. F., Versuche und Beobachtungen über die Bastardzeugung im Pflanzenreich. Stuttgart. 1849.
5. GARDNER, F. E., and KRAUS, E. J., Histological comparison of fruits developing parthenocarpically and following pollination. BOT. GAZ. **99**:355–376. 1937.
6. GUSTAFSON, F. G., Inducement of fruit development by growth promoting chemicals. Proc. Nat. Acad. Sci. **22**:628–636. 1936.
7. ———, Parthenocarpy induced by pollen extracts. Amer. Jour. Bot. **24**:102–107. 1937.
8. HAMNER, K. C., and KRAUS, E. J., Histological reactions of bean plants to growth-promoting substances. BOT. GAZ. **98**:735–807. 1937.
9. KRAUS, E. J., BROWN, NELLIE A., and HAMNER, K. C., Histological reactions of bean plants to indoleacetic acid. BOT. GAZ. **98**:370–420. 1936.

10. KRAUS, E. J., and MITCHELL, J. W., Histological and physiological responses of bean plants to alpha naphthalene acetamide. BOT. GAZ. **101**:204–225. 1939.
11. ———, Histological reactions of bean plants to l-tryptophane. BOT. GAZ. **102**:602–622. 1941.
12. LAIBACH, F., and KORNMANN, P., Zur Methodik der Wuchsstoffversuche. Planta **19**:482–484. 1933.
13. LA RUE, C. D., The effect of auxin on abscission of petioles. Proc. Nat. Acad. Sci. **22**:255–259. 1936.
14. MASSAST, J., Sur la pollination sans fecondation. Bull. Jard. Bot. (Bruselles) **1**:85–95. 1902.
15. MILLARDET, A., Rev. de viticulture **16**:677–680. 1901.
16. MILLER, E. C., Plant physiology. New York. 1931.
17. MITCHELL, J. W., A method of measuring respiration and carbon fixation of plants under controlled environmental conditions. BOT. GAZ. **97**:376–387. 1935.
18. MITCHELL, J. W., and MARTIN, W. E., Effect of indoleacetic acid on growth and chemical composition of etiolated bean plants. BOT. GAZ. **99**:171–183. 1937.
19. MITCHELL, J. W., and STEWART, W. S., Comparison of growth responses induced in plants by naphthalene acetamide and naphthaleneacetic acid. BOT. GAZ. **101**:410–427. 1939.
20. MYERS, R. M., Effect of growth substances on the absciss layer in leaves of *Coleus*. BOT. GAZ. **102**:323–338. 1940.
21. VAN OVERBEEK, J., Auxin distribution in seedlings and its bearing on the problem of bud inhibition. BOT. GAZ. **100**:133–166. 1938.
22. WENT, F. W., and THIMANN, K. V., Phytohormones. New York. 1937.
23. YASUDA, S., On the behavior of the pollen tube in the production of seedless fruits by interspecific pollination. II. Jap. Jour. Genetics **9**:118–124. 1934.

INFLUENCE OF LOCALIZED LOW TEMPERATURE ON BILOXI SOYBEAN DURING PHOTOPERIODIC INDUCTION

H. A. BORTHWICK,[1] M. W. PARKER,[2] AND P. H. HEINZE[3]

(WITH TWO FIGURES)

Introduction

Numerous studies dealing with the effect of the interaction of photoperiod and temperature upon flowering have recently been made (**3, 4, 5, 6, 7, 8, 9**). In some of these studies the experimental treatments were continued for several weeks whereas in others they were restricted to durations of only a few days. In general, the response to photoperiod has been markedly influenced by variations in temperature.

In Biloxi soybean plants low temperature during photoperiodic induction resulted in a decrease in floral initiation (**7**). In this study, and in most others of similar nature, the entire plant was subjected to the various treatments imposed, and so it was not clear just what particular process or processes were affected.

Low temperature has been shown to produce a change in the metabolism of the leaf cells (**7**), and since the leaf is known to be the seat of photoperiodic stimulation in the plant, such changes could account for the different flowering responses observed. It is also possible that low temperature may influence the transfer of materials from the leaves to the growing points. Such effects have been demonstrated in other plants (**2**) and might be expected in the soybean. More or less complete inhibition of the transfer of food materials or of any special flower-inducing substance that may be formed in the leaves would probably result in a difference in the extent of flower-bud initiation induced by a given treatment. Reduction in flower-bud formation could also result from the action of low temperature upon the meristems themselves, although these growing points may be supplied with adequate amounts of food and growth-regulating substances.

The data presented here deal particularly with the influence of low temperature applied to the growing points of stems and to the petioles of leaves during the period of photoperiodic induction.

Material and methods

In preliminary attempts to chill the petioles of leaves several methods were tried before a satisfactory one was developed. In the method finally used, cold

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water was circulated through a glass cylinder (fig. 1) sealed about the petiole. It was in actual contact with the petiole and produced a uniform temperature throughout the tissue inclosed in the cell.

The cooling cells were made of glass tubing approximately $1\frac{1}{2}$ inches in diameter and 2 inches in length. The cells were fitted with rubber stoppers on which shoulders were cut in such a way that they could be inserted for $\frac{1}{4}$ inch into each end of the cell, thereby providing a uniformly effective cooling length of $1\frac{1}{2}$ inches.

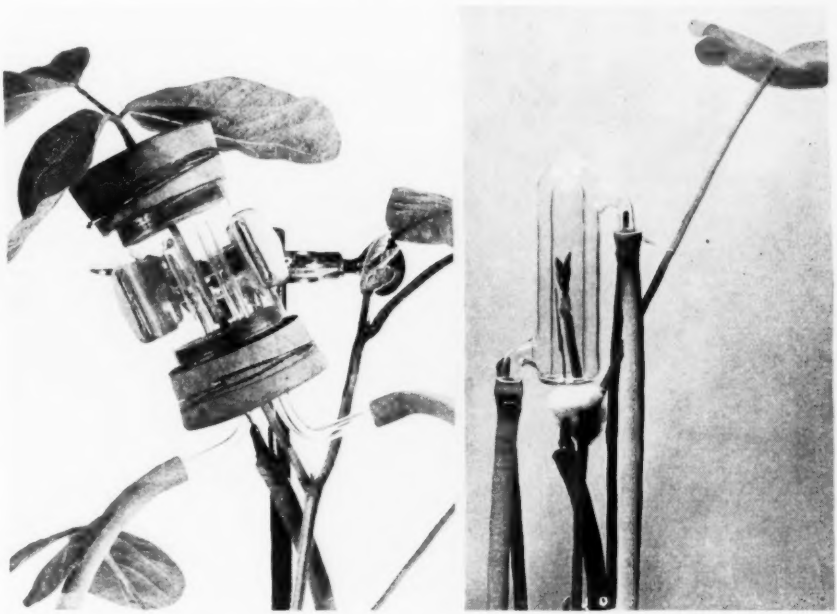

FIG. 1.—Apparatus for localized cooling. Petiole cooler on left and tip cooler on right

The stoppers were split to a hole in the center so that they could be placed about the petiole. The lower stopper was fitted with inlet and outlet tubes for circulation of the water and with a well for a thermocouple.

The cooling cells were placed on the petiole by loosely rolling the three leaflets of a leaf and passing them through the glass cell. The stoppers were spread open and grafting wax of a low melting point was placed in the hole that received the petiole. When the petioles were in place the stoppers were gradually pressed into position. The stoppers were bound together by means of wire, thus insuring an effective seal between the petiole and the stopper.

Double-walled glass cells were devised to cool the stem tips. These cells (fig. 1)

consisted of two glass tubes sealed together in such a way that the stem tip with its terminal bud could be inserted from the base. Inlet and outlet tubes were sealed to the sides. The effective thickness of the layer between the two tubes was approximately 5 mm., and the effective length of the cells was 8.5 cm. The stem tip and a thermocouple wire were inserted into the cells when the apparatus was set up, and a cotton plug was placed in the bottom. In figure 1 this plug has been partly withdrawn to show the lower part of the cell.

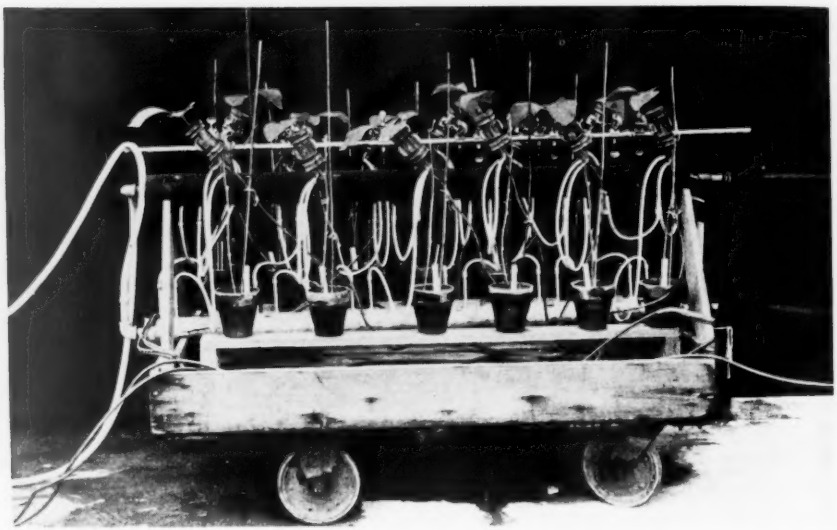

FIG. 2.—General view of plants and apparatus for typical petiole-cooling experiment. A similar truck was used for tip-cooling cells.

An insulated tank containing 30 gallons of water and a refrigerator to cool it were installed on a warehouse truck. The water was circulated to the petiole and tip-cooling cells through manifolds mounted on two other trucks (fig. 2).

Temperatures of each cell and of the water in the tank were recorded three or more times daily. Temperatures of the cells were obtained from thermocouples read with a portable Leeds and Northrup potentiometer; those of the tank were taken with a mercury thermometer. The temperatures reported for the individual experiments varied $\pm 2^{\circ}$ C. for short periods during the day, but were very close to the temperatures indicated during the night.

The various lots of plants were grown in the greenhouse during the months of November, 1940, to April, 1941, inclusive. They received the natural photoperiods then prevailing and Mazda light from sundown until midnight. The plants varied in age from 42 to 65 days at the beginning of the treatments.

In the experiments involving cooling of the stem tips, all large leaves were removed from experimental and control plants except the one youngest fully-expanded leaf on each plant. The two partially-expanded leaves immediately above were also removed in all experiments, and in a few cases three such leaves were removed. All buds were removed from the plants except those in the axils of the unexpanded leaves remaining at the tip of the stem. These buds were within the cooling cells and were therefore subjected to the temperature maintained during any particular experiment. The only part of the plant capable of flower-bud initiation was therefore restricted to the region where the temperature was controlled.

Defoliation for the petiole-cooling experiments was done as described for the tip-cooling experiments, except that in some experiments the two most recently fully-expanded leaves were left on the plant. No buds were removed from plants of the petiole-cooling experiments.

Induction treatments consisting of four 8-hour photoperiods were used in all the experiments. In those involving plants with single leaves the short days were obtained by placing the group of three trucks in a dark chamber at 4:00 P.M. and removing them at 8:00 A.M. In those experiments involving plants with two leaves, one on short and one on long photoperiod, the short photoperiod was provided by means of black cloth bags as described previously (1). Long day was provided by extending the natural photoperiod with Mazda light. In these experiments the trucks were left in the greenhouse. The control plants were defoliated and disbudded in exactly the same way as the experimental plants with which they were associated.

After the treatments were completed, the plants were returned to long photoperiods and dissected 2–3 weeks after the start of the induction treatment.

Experimental results

Petiole-cooling experiments were begun in November, 1940, and continued through April, 1941. During this time a wide range of environmental conditions, such as variability in light intensity and greenhouse temperature, was encountered. The age and general condition of the plants also varied from one experiment to another. In all the experiments, however, it was possible to demonstrate a retarding influence on floral initiation when low temperature was applied (table 1).

The first series of tests dealt with plants which had been defoliated to one fully-expanded trifoliate leaf and included experiments conducted at various temperatures. These single-leaved plants received an induction treatment consisting of four 8-hour photoperiods. When the temperature of the petiole was maintained at 3° C. during the induction period, the production of flower primordia was greatly inhibited. In three experiments run during November, only one plant of ten in each lot formed flower primoria. In the other experiment, in which the petiole was maintained at 3° C., eight of ten plants formed primordia but the total number of

flower buds was only fourteen, while similar control plants produced thirty-seven. When the temperature of the petiole was raised to 6° C., eight of eleven plants produced flower primordia. At this temperature the cooled lot produced a total of twenty-five flower buds while the control plants produced forty-six.

When water at 25° C. was circulated around the petioles, all the plants formed flower primordia. The total number formed on the ten plants was twenty-six.

TABLE 1

FLORAL INITIATION AS AFFECTED BY COOLING PETIOLES OF BILOXI SOYBEAN DURING PHOTOPERIODIC INDUCTION

TREATMENT STARTED	NO. OF NODE WHERE LEAF REMAINED	TEMPERATURE OF WATER (° C.)	PLANTS WITH COOLING CELLS ON PETIOLES					PLANTS WITHOUT COOLING CELLS ON PETIOLES				
			PLANTS PER LOT	PLANTS WITH FL. PR.	FL. PR. PER LOT	TOTAL NO. OF NODES	INCREASE IN LENGTH OF LEAF* (CM.)	PLANTS PER LOT	PLANTS WITH FL. PR.	FL. PR. PER LOT	TOTAL NO. OF NODES	INCREASE IN LENGTH OF LEAF* (CM.)
11/8/40	5	3	10	1	2	17.9	0.32	10	6	8	18.7	1.79
11/12/40	6	3	10	1	1	19.2	0.40	10	10	23	19.9	2.13
11/22/40	6	3	10	1	2	18.8	0.49	10	8	17	19.4	0.98
2/6/41	6	3	10	8	14	21.3	0.07	10	10	37	22.4	0.58
2/18/41	6	6	11	8	25	22.8	1.04	12	12	46	23.7	1.57
4/28/41	5	7	12	7	15	19.8	2.37	12	12	47	20.7	2.84
11/18/40	7	25	10	10	26	20.9	1.27	10	10	32	21.0	0.88
11/26/40	4, 5	3	10	1	1	19.5	0.96	10	10	29	19.9	1.12
3/17/41	5, 6	3	12	2	2	21.2	1.30	12	11	21	21.7	1.36
12/2/40	5, 6	3	10	1	4	21.1	1.34	10	10	23	21.1	0.99
2/25/41	6, 7	3	12	4	10	22.0	0.70	12	11	32	22.4	0.72
4/21/41	8, 9	5	12	7	15	26.2	0.88	12	12	47	26.6	0.86
3/24/41	5, 6	6	12	8	15	22.2	4.09	12	12	32	22.8	4.69
3/3/41	6, 7	6	12	9	20	24.6	1.42	12	12	43	23.5	0.93
4/14–/41	7, 8	10	12	12	55	25.0	4.16	12	12	64	25.2	3.29

* Center leaflet of leaf at base of terminal bud measured.

Similar plants receiving the same photoinductive treatment but not having cooled petioles produced thirty-two flower primordia on ten plants. This experiment indicates that the mere presence of water around the petiole does not interfere with movement of the flower-forming stimulus from the leaf to growing points.

In experiments at 3° C. the increase in length of the center leaflet of the lowermost leaf in the terminal bud was much greater in the controls than in the plants with the cooled petioles. The total number of nodes in the main axis of the control plants was also greater in these experiments than in the plants with cooled petioles. These facts indicate that growth during the photoinductive treatment was greatly reduced.

Since all the food for the growth of these plants was furnished by a single leaf, the petiole of which was cooled, the reduced growth relative to that of the controls was to be expected. It has been shown (2) that this procedure greatly reduces the translocation of carbohydrates. Since apparently very few structures were initiated during the induction period of these experiments, it could not definitely be shown that a flower-forming stimulus was not present in the growing points. To determine whether or not such a stimulus had passed through the cooled petiole, it was necessary to insure the production of new structures upon which the stimulus might act. This was provided by leaving on the plant a second leaf which was not cooled and which was kept on long photoperiod. This leaf supplied food to the growing point, but—on the basis of previous experiments—did not influence the capacity of the remainder of the plant to form flower primordia. The uppermost leaf was given short photoperiods and its petiole was cooled to various temperatures as in the previous experiments. The data with two-leaved plants in table 1 show that growth, as measured by increase in leaf length and total number of nodes on the main axis, was approximately the same whether or not the petioles of the uppermost leaf were cooled. This indicates that growth of the terminals was maintained at a rate comparable with that of the controls. In all the experiments at this temperature the total number of flower primordia produced on the plants with cooled petioles was much less than on the controls, indicating that the movement of the flower-forming stimulus was definitely reduced when the petioles were cooled to 3° C.

When the temperature was increased to 5° or 6° C., more of the plants with cooled petioles produced flower buds. At 10° C. all the plants in the cooled lot produced flower buds, and the total number approached the total number produced by the controls.

In the tip-cooling experiments low temperature applied to the stem terminals resulted in marked reduction in flower-bud initiation (table 2). These plants were disbudded at all nodes, except those in the terminal buds, which were surrounded by the cooling cells. All the leaves, except the one most recently fully expanded and the small unexpanded leaves in the terminal bud, were removed. Control plants were disbudded and defoliated in the same manner.

When the terminal bud was cooled to 3° C. no flower primordia were formed on two lots of eleven plants each. All the twenty-two control plants of these two lots produced flower primordia. One lot formed twenty-five and the other sixty-three. In another lot cooled to the same temperature seven of the eleven plants formed a total of twelve flower buds. The eleven control plants all initiated flower primordia and formed a total of forty buds.

At a temperature of 6° C., twenty-one of the twenty-two plants in two experiments produced a total of forty-one flower buds. All the twenty-two control

plants of these two lots formed flower primordia. The total number formed, however, was more than twice as many as on the tip-cooled plants.

At 10° C. all plants in both the tip-cooled and the control lots initiated flower buds. The number formed by the controls was somewhat less than twice the number formed by the tip-cooled plants. In general, smaller differences in the extent of flower-bud initiation between tip-cooled and control lots resulted as temperature increased.

Increase in length of the leaflets measured was very slight at all temperatures. The increase in the controls, although variable from one experiment to another,

TABLE 2

FLORAL INITIATION AS AFFECTED BY COOLING STEM TIPS OF BILOXI SOYBEAN DURING PHOTOPERIODIC INDUCTION

Treatment started	Temperature of water (° C.)	Plants with terminal coolers					Plants without terminal coolers				
		Plants per lot	Plants with fl. pr.	Fl. pr. per lot	Total no. of nodes	Increase in length of leaf*	Plants per lot	Plants with fl. pr.	Fl. pr. per lot	Total no. of nodes	Increase in length of leaf* (cm.)
2/25/41	3	11	7	12	22.0	0.02	11	11	40	22.5	0.90
3/17/41	3	11	0	.....	20.4	.05	11	11	25	21.9	2.07
4/21/41	4	11	0	.....	25.4	.07	11	11	63	27.0	1.07
2/18/41	6	11	11	25	22.3	.04	11	11	51	23.8	1.22
3/3/41	6	11	10	16	22.2	.03	11	11	39	23.4	1.28
4/14/41	10	11	11	32	22.1	0.21	11	11	57	24.6	6.96

* Center leaflet of leaf at base of terminal bud measured.

was much greater than that of the corresponding tip-cooled lots. Less striking differences but of the same trend occurred in the total number of nodes formed. The temperatures reported in these experiments all appear—on the basis of the type of measurements made—to have inhibited growth to about the same degree. Flower-bud formation, however, was much more markedly supressed at the lower than at the higher temperatures.

Discussion

It is known that the leaves of plants are the organs of photoperiodic perception, and that the formation of flower buds resulting from photoperiodic stimulation occurs in any growing points of the stem. This necessitates the transport of the stimulus from the leaves to the growing points. The influence of temperature on the production of flower buds, therefore, could be exerted in at least three ways:

(1) upon the organs of perception (leaves); (2) upon the structures and processes involved in translocation of the stimulus from these organs to the growing points; or (3) upon the growing points themselves. Any one or any combination of these could affect the differentiation of flower buds.

In the case of the petiole-cooling experiments, the effects were limited to the structures and processes of translocation, whereas in the case of the tip-cooling experiments the apical meristems were primarily subjected to the influence of low temperature, although the structures and functions concerned with translocation were also influenced to the extent that the stem was inclosed in the tip cooler. In either case the organs of perception were uninfluenced by the conditions of low temperature, since they were subjected only to the conditions prevailing in the greenhouse. The influence of temperature upon the processes in the leaf, in so far as they pertain to flower-bud formation, are being subjected to further investigation.

When the petioles were cooled to 3° C., floral initiation was greatly suppressed or completely inhibited. At 10° C., however, most of the plants formed flower buds, although less extensively than the controls. In the tip-cooling experiments it was also found that flower-bud formation could be greatly limited by a very low temperature, but at 10° C. floral initiation was fairly abundant on all the plants. Thus only when the processes involved in translocation and in formation of new structures were subjected to extremely low temperatures was flower-bud formation markedly suppressed.

In previous experiments (**7**) with soybean, in which whole plants were subjected to low temperature, it was found that at 55° F.–12.8° C. no flower primordia were formed. On the basis of the present experiments, their failure to form at that temperature was not a result of limitation of the transport of the flower-forming stimulus nor of the production of new structures at the growing points.

Summary

1. The influence of low temperature in suppressing flower-bud initiation in Biloxi soybean has been considered from three points of view: its influence upon the differentiation of flower buds from the meristems; its influence on translocation of a flower-forming stimulus from the leaves to the growing points; and its influence upon those processes in the leaf that bring about flower-bud formation. Data are presented on the first two of these points; the last is being subjected to further investigation.

2. Fewer flower buds were formed in response to a 4-day induction treatment on plants bearing a single leaf if the terminal buds or the petioles were cooled to 3° C. than if they were not cooled. When the temperature was raised to 10° C. the inhibiting effect on flowering decreased in both types of experiments, but even at

this temperature only slight growth of the terminals occurred during the induction treatment.

3. Petiole-cooling experiments involving plants with two leaves indicate that inhibition of flowering resulted from the influence of low temperature on the transport of a flower-forming stimulus.

U.S. HORTICULTURAL STATION
BELTSVILLE, MARYLAND

LITERATURE CITED

1. BORTHWICK, H. A., and PARKER, M. W., Photoperiodic perception in Biloxi soybeans. BOT. GAZ. **100**:374–387. 1938.
2. CURTIS, O. F., Studies on solute translocation in plants. Experiments indicating that translocation is dependent on the activity of living cells. Amer. Jour. Bot. **16**:154–168. 1929.
3. KNOTT, J. E., The effect of temperature on the photoperiodic response of spinach. Cornell Agr. Exp. Sta. Memoir 218. 1939.
4. LONG, E. M., Photoperiodic induction as influenced by environmental factors. BOT. GAZ. **101**:168–188. 1939.
5. MANN, L. K., Effect of some environmental factors on floral initiation in *Xanthium*. BOT. GAZ. **102**:339–356. 1940.
6. NAYLOR, A. W., Effects of some environmental factors on photoperiodic induction of beet and dill. BOT. GAZ. **102**:557–575. 1941.
7. PARKER, M. W., and BORTHWICK, H. A., Effect of variation in temperature during photoperiodic induction upon initiation of flower primordia in Biloxi soybean. BOT. GAZ. **101**:145–167. 1939.
8. ROBERTS, R. H., and STRUCKMEYER, B. ESTHER, The effects of temperature and other environmental factors upon the photoperiodic responses of some of the higher plants. Jour. Agr. Res. **56**:633–677. 1938.
9. SNYDER, W. E., Effect of light and temperature on floral initiation in cocklebur and Biloxi soybean. BOT. GAZ. **102**:302–322. 1940.

EFFECTIVENESS OF TRYPTOPHANE MIXTURES AS GROWTH REGULATORS

WILLIAM S. STEWART[1]

(WITH TWO FIGURES)

It has been shown (1) that a lanolin mixture containing 2 per cent by weight of l-tryptophane is capable of inducing gall formation when applied to the cut surface of decapitated bean seedlings. In an attempt to extend the experiments reported, another lot of tryptophane-lanolin mixture was tried. It was observed that gall formation took place much more slowly, but that when the original mixture made up in 1939 was again tried, the plants responded to it in the same way as they had done previously. About 10 cu. mm. of each of the two mixtures was then applied unilaterally, 15 mm. below the tip of *Avena* coleoptiles which were grown and decapitated twice as they are for the *Avena* test (4). The result was that the original 1939 mixture induced a very great bending response whereas the second lot caused no bending whatsoever (fig. 1).

It was then decided to try a more extensive series of tests using l-tryptophane from several sources, made up both as mixtures with lanolin and in aqueous solution mixed with agar. Five lots were used as follows: the original sample (1) furnished by the Eastman Kodak Company in 1939; two other samples (2, 3) from the same company, one secured in 1940 and the other in 1941; a fourth sample (4) furnished by the Pfanstiehl Company in 1941; and a fifth (5) secured from Merck and Company in 1941.

When the last four samples were stirred directly with lanolin and these mixtures applied to *Avena* coleoptiles, there was no subsequent curvature. It was observed, however, that the crystals of tryptophane were not very evenly dispersed throughout the lanolin. To secure a more uniform mixture, another method of preparing the mixtures was used. To 100 mg. of each of the samples of tryptophane, 0.2 cc. of water was added, 5 gm. of lanolin was melted and this quantity poured into each of the respective vials containing the tryptophane. These were then heated in a water bath at 100° C. and stirred vigorously. After several minutes the vials were removed from the water bath and the stirring continued until the mixture solidified. Microscopic examination showed that there was a much more nearly uniform dispersion of the tryptophane in the lanolin and the crystals were much smaller (fig. 2). When these mixtures were applied to decapitated bean plants, tumors were formed to the same degree and in the same length of time in all instances as with the East-

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man no. 1 sample. One-gm. aliquots of each of these samples of tryptophane were dissolved in 250 cc. of water and autoclaved for 20 minutes at 120-lb. pressure. The water was then evaporated off on a steam bath. The residue was dried over night at 80° C. Each of the residues was made up as a 2 per cent lanolin mixture as just described, and in water solution. When the lanolin mixtures of these autoclaved samples were tested on beans, the results were similar to those obtained with the lanolin mixtures last described.

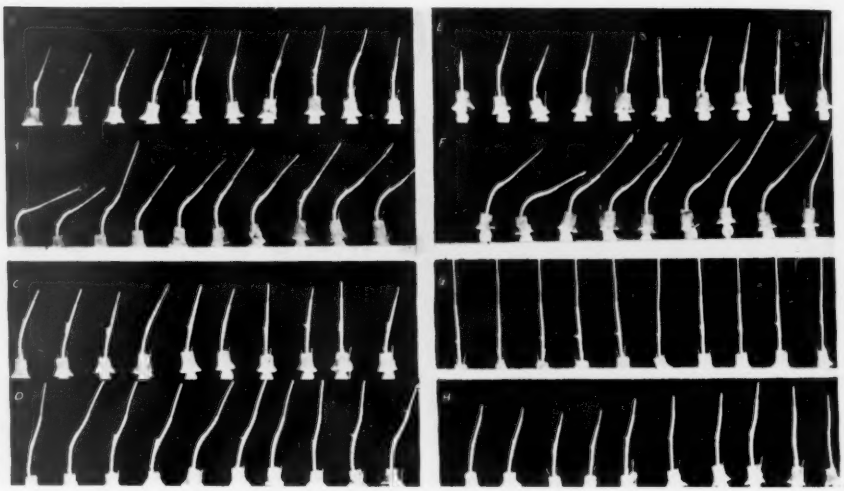


FIG. 1.—Response of *Avena* coleoptiles to unilateral applications of 2% mixtures of tryptophane in lanolin: *A*, Eastman 1939, original mixture, 1½ hours after application; *B*, same after 20 hours; *C*, Eastman 1939, autoclaved, 1½ hours; *D*, same after 20 hours; *E*, Eastman 1941, very finely divided and well dispersed mixture, 1½ hours; *F*, same after 20 hours; *G*, Eastman 1939, large crystals not well dispersed, 20 hours; *H*, Pfanstiehl 1941, 1½ hours, sample prepared by adding 0.2 cc. water and melted lanolin, then stirring vigorously until cooled.

A tryptophane-lanolin mixture still more finely divided and uniformly dispersed was obtained by dissolving 100 mg. of tryptophane, lot 3, in about 5 cc. of hot water. This solution was then added to 5 gm. of lanolin heated in an oil bath to 120–140° C. The water was evaporated off under reduced pressure. Microscopic examination showed no large distinguishable crystals (fig. 2). Application of this mixture to bean plants resulted in the formation of tumors somewhat more rapidly than any of the other mixtures previously tried.

When the original tryptophane-lanolin mixture prepared in 1939 was applied as a narrow band around the first internodes of young bean seedlings, as had been done by MITCHELL and WHITEHEAD (2) with a pollen extract-lanolin mixture,

there was elongation of such internodes to a greater degree than in control plants. One week following treatment, when elongation had practically ceased, those internodes to which tryptophane had been applied were significantly longer than the controls. All the plants treated with the tryptophane-lanolin mixtures made up

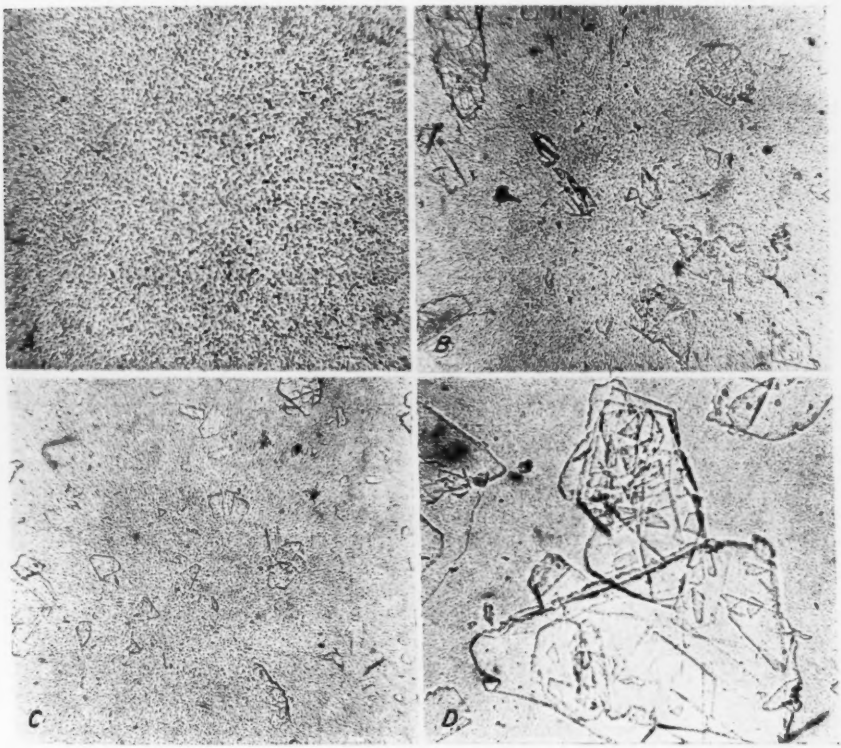


FIG. 2.—Two per cent mixtures of tryptophane in lanolin: *A*, same as *E* in fig. 1; *B*, Eastman 1941 prepared as *H* in fig. 1; *C*, original mixture, Eastman 1939; *D*, same as *G* in fig. 1. All ×100.

by adding 0.2 cc. of water to the tryptophane before mixing were approximately 45 per cent longer than controls. Application of the mixture in which the tryptophane was finely divided resulted in an 80 per cent increase in length of the first internode. In this particular experiment elongation following application of corn pollen extract-lanolin mixture was 98 per cent.

The standard *Avena* test showed that an aqueous solution of Eastman 1939 tryptophane in agar caused the greatest amount of growth promotion. Pfanstiehl tryptophane 1941, lot 5, caused practically none (table 1). Results similar to those

recorded in the table were obtained with tryptophane autoclaved as described. Thus heating tryptophane, as in preparing the lanolin mixtures, apparently does not increase its growth-promoting properties.

All the lanolin mixtures of tryptophane containing 0.2 cc. of water, including the Pfanstiehl mixture, resulted in growth promotion when applied as described to *Avena* coleoptiles. The increased growth was apparent $1\frac{1}{2}$ hours after application (fig. 1; table 1).

This discrepancy in the action of the Pfanstiehl tryptophane—when applied in lanolin to the side of a coleoptile and when applied as in the *Avena* test at the top of the coleoptile in a water solution in agar—may be due to several differences in

TABLE 1

RESPONSE OF AVENA COLEOPTILES TO TRYPTOPHANE $1\frac{1}{2}$ AND 20 HOURS AFTER APPLICATION. AS DEGREES CURVATURE PER TWELVE COLEOPTILES WITH STANDARD ERROR

SOURCE	STANDARD AVENA TEST 50 MG./L. $1\frac{1}{2}$ HOURS	UNILATERAL APPLICATION OF 2% LANOLIN MIXTURE	
		$1\frac{1}{2}$ HOURS	20 HOURS
Eastman 1939	10.5±0.7	11.7±1.6	17.6±1.6
Eastman 1940	6.5±1.3	9.3±0.9	19.9±1.7
Eastman 1941	6.0±0.3	5.5±1.5	15.2±2.6
Merck 1941	6.5±1.2	6.5±1.6	13.7±1.7
Pfanstiehl 1941	1.0±0.4	7.5±1.5	14.6±1.5
Eastman 1939, original lanolin mixture	.....	16.8±1.2	53.9±4.2
Eastman 1941, finely dispersed lanolin mixture	.....	11.1±2.1	53.1±3.3

the methods of application: (*a*) tryptophane applied in lanolin directly to the region of elongation of the *Avena* coleoptile does not have to travel downward 15 mm. or so to reach the presumed site of action; (*b*) lanolin itself has very rapid intercellular penetration (3), and thus might carry tryptophane along with it directly to the cells in which it was to act; (*c*) the area of contact of the lanolin on the side of an *Avena* coleoptile is much greater than that of an agar block on the top cut surface.

Unilateral application to *Avena* coleoptiles of the lanolin-tryptophane mixture, in which that tryptophane was finely divided[4], resulted in growth-promotion curvatures as great as those from the original 1939 mixture.

It is obvious that in preparing mixtures of lanolin and tryptophane—and probably other synthetic growth-regulating substances as well—considerable care should be taken to assure uniform, finely divided dispersion of the substance in lanolin, and that results may vary greatly, depending upon the character of the

mixture. Whether the tryptophane is changed in the process of preparation, or whether there may be other substances present in the original samples of tryptophane, remains to be determined. If other substances are present in such samples, they apparently occur in all that were used.

U.S. HORTICULTURAL STATION
BELTSVILLE, MARYLAND

LITERATURE CITED

1. KRAUS, E. J., Histological reactions of bean plants to l-tryptophane. BOT. GAZ. **102**:602–622. 1941.
2. MITCHELL, J. W., and WHITEHEAD, MURIEL R., Responses of vegetative parts of plants following application of extract of pollen from *Zea mays*. BOT. GAZ. **102**:770–791. 1941.
3. SCOTT, FLORA M., Anatomy of auxin treated etiolated seedlings of *Pisum sativum*. BOT. GAZ. **100**:167–185. 1938.
4. WENT, F. W., and THIMANN, K. V., Phytohormones. New York. 1937.

MEGASPOROGENESIS AND DEVELOPMENT OF THE MEGAGAMETOPHYTE IN NOTHOSCORDUM BIVALVE

MARY LOUISE ECKLES

(WITH NINE FIGURES)

Introduction

The genus *Nothoscordum*, a member of the Liliaceae, is closely related to *Allium;* in fact, SMALL (**6**) places it in the Alliaceae. Over twenty species have been reported, only two of which occur in the United States. *Nothoscordum bivalve* (L.) Britton is found growing in sandy soil, from Virginia to Nebraska and southward into Mexico.

Several types of development of the megagametophyte have been reported in the Liliaceae. SCHNARF (**5**) and MAHESHWARI (**4**) report the occurrence of the *Scilla*-type, the *Allium*-type, the *Adoxa*-type, the *Fritillaria*-type, and even one case of the *Oenothera*-type—in *Clintonia borealis* as reported by SMITH (**7**). In addition, the so-called normal type is of frequent occurrence.

It was thought that megasporogenesis and the development of the megagametophyte in *Nothoscordum bivalve* might conform to one of the aberrant types characterizing other members of the family. However, it was found to follow the more typical development.

Few studies have been made of *N. bivalve*. Polyembryony in *N. fragrans* has been reported by several workers, STRASBURGER (**8**) being one of the earliest. KOERPERICH (**3**) investigated mitosis in *N. fragrans* and found the somatic chromosome number to be 16. ANDERSON (**1**) found the haploid number in *N. bivalve* to be 9. BEAL (**2**) described microsporogenesis and chromosome behavior in *N. bivalve* and confirmed ANDERSON's count. Megagametophyte formation in this species has not been described.

Material and methods

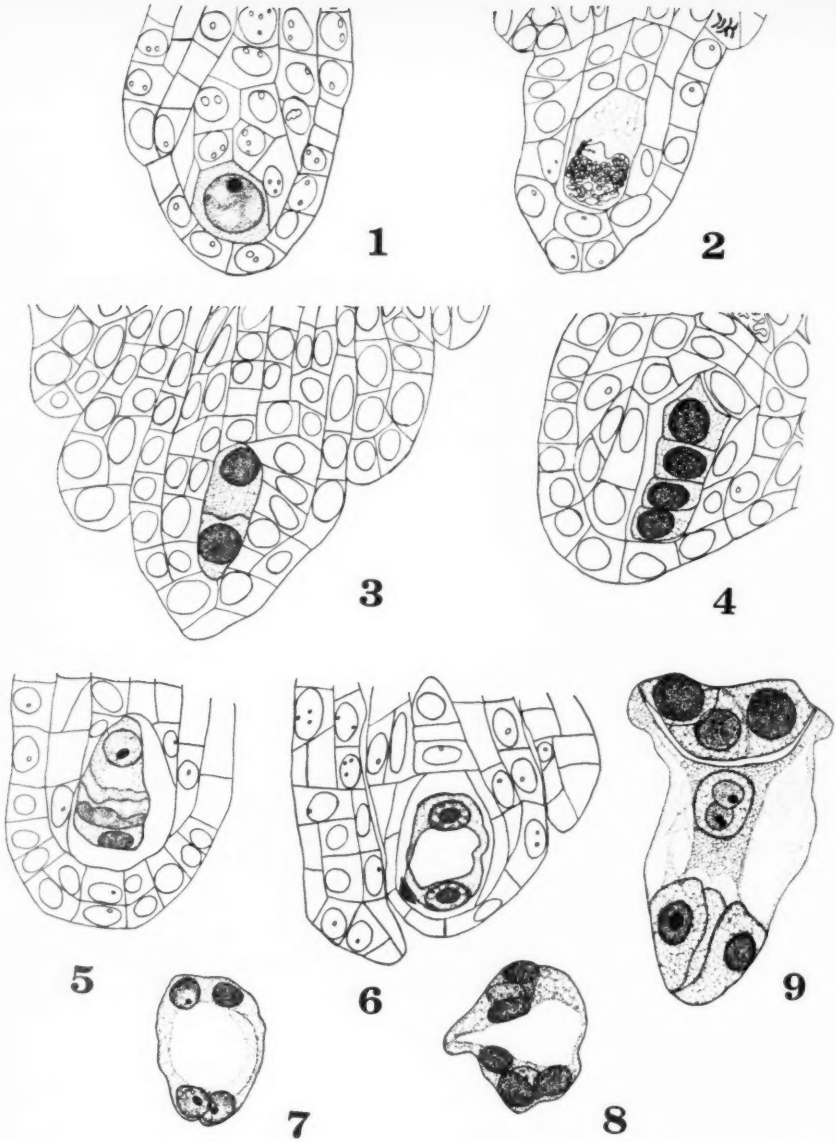
The material used in this study was collected on the campus of Mississippi State College. Flowers of various ages were collected, ranging from buds just emerging from the bulb to flowers that had been open several days. In all the early stages the entire floral structure, with only the two bracts removed, was fixed; in the older flowers only the pistil was used. To insure good penetration of the fixative, the tips of the buds were cut off, as was most of the style in the older flowers. The material was placed in Randolph's modification of Navashin's

fixative, and since it did not sink readily, a water-pressure pump was used. After washing in water, dehydrating in various grades of alcohol, and clearing with chloroform, the material was imbedded in paraffin. Longitudinal sections were cut 10–25 μ in thickness. Some of these were stained with Flemming's triple stain, some in iron-alum haematoxylin, and some in iron-alum haematoxylin with orange gold in clove oil as a counter-stain. The last-named proved the most satisfactory.

Observations

The archesporial cell functions as the megaspore mother cell. This is easily distinguished from other nucellar cells by its large size and its hypodermal position. In section, the nucleus fills nearly three-fourths of the cell and contains one to several nucleoli of varying size. The cytoplasm contains numerous small vacuoles but no large ones (fig. 1). The cell elongates somewhat before undergoing the meiotic divisions to form a row of four. The nucleus during synizesis of the first meiotic divisions is shown in figure 2. After the first division the two daughter nuclei are separated by a distinct wall (fig. 3). A second nuclear and cell division form the four megaspores (fig. 4). The one nearest the chalazal end of the ovule is destined to be the functional megaspore, and from the first it is the largest of the four. As the functional megaspore begins to enlarge, the other three disintegrate, the one next to the functional megaspore initiating the disintegration and the others following in succession. As the non-functional spores disappear, the functional one enlarges and its cytoplasm becomes greatly vacuolated (fig. 5). The disappearance of the disintegrating megaspores is not complete until the nucleus of the functional megaspore has divided to form two nuclei, one of which migrates to each end of the cell (fig. 6). The megagametophyte then begins to enlarge at the expense of the surrounding cells, whose remnants appear as darkly staining streaks. In most cases a rather large central vacuole, formed by coalescence of many small vacuoles, appears between the two nuclei, each of which contains a conspicuous nucleolus. Each nucleus divides so that four nuclei, two at each end, are formed (fig. 7). The developing gametophyte continues to enlarge and the four nuclei divide simultaneously. Of the eight nuclei now present (fig. 8), two, one from each end, migrate toward the center of the gametophyte. These polar nuclei meet but do not fuse. Cell delimitation takes place so that seven cells are formed, each with a single nucleus—with the exception of the primary endosperm cell which contains the two polar nuclei.

The mature megagametophyte (fig. 9) thus consists of a central primary endosperm cell containing the two polar nuclei; three antipodal cells at the chalazal end, and an egg and two synergids at the micropylar end. The antipodal cells are closely grouped. Although smaller than the synergids, they contain large nuclei which apparently lack nucleoli. The nuclei in the primary endosperm cell are



FIGS. 1–9.— Figs. 1–4, formation of megaspores: Fig. 1, megaspore mother cell imbedded in nucellus. Fig. 2, same during synizesis. Fig. 3, two cells formed by first division of megaspore mother cell. Fig. 4, row of four megaspores, the chalazal spore the functional one. Figs. 5–9, development of megagametophyte from megaspore: Fig. 5, functional megaspore distinct; other three disintegrating. Fig. 6, 2-nucleate stage following division of the functional megaspore nucleus. Fig. 7, 4-nucleate stage. Fig. 8, early 8-nucleate stage following simultaneous division of nuclei in fig. 7. Fig. 9, mature megagametophyte showing three antipodal cells, primary endosperm cell with its two nuclei, two synergids, and a little of the egg cell.

small, each containing one or more nucleoli. The synergids are large and somewhat oval in section, each with a large nucleus containing a nucleolus. The egg lies between the two synergids and projects somewhat into the central region of the megagametophyte. The cytoplasm of the egg is vacuolated and not very dense, and the nucleus, usually containing one nucleolus, does not stain deeply.

About the time that the megaspore mother cell begins to enlarge preparatory to division, the inner integument first appears as a slight bulge (fig. 1). About the time of the first division, the second or outer integument begins to form (fig. 3).

Summary

1. The archesporial cell serves as the megaspore mother cell.

2. From the megaspore mother cell by two divisions a row of four megaspores is formed.

3. The chalazal megaspore is the functional one; the other three disintegrate, beginning with the one next to the functional megaspore.

4. Development of the megagametophyte follows the typical course; three nuclear divisions result in the formation of eight nuclei, of which two function as polar nuclei, the others becoming the nuclei respectively of the egg, two synergids, and three antipodal cells.

The writer is grateful to Professors J. C. McKee and J. F. Locke of Mississippi State College and Professor C. E. Allen of the University of Wisconsin for their helpful suggestions and criticisms.

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LITERATURE CITED

1. Anderson, E., The chromosome complements of *Allium stellatum* and *Nothoscordum bivalve*. Ann. Missouri Bot. Gard. **18**:465–468. 1931.
2. Beal, J. M., Microsporogenesis and chromosome behavior in *Nothoscordum bivalve*. Bot. Gaz. **93**:278–295. 1932.
3. Koerperich, J., Etude comparative du noyau, des chromosomes et de leurs relations avec le cytoplasme (*Nothoscordum*, *Eucomis*, *Beschorneria*). La Cellule **39**:309–398. 1930.
4. Maheshwari, P., A critical review of the types of embryo sacs in angiosperms. New Phytol. **36**:359–417. 1937.
5. Schnarf, K., Contemporary understanding of embryo-sac development among angiosperms. Bot. Rev. **2**:565–585. 1936.
6. Small, J. K., Manual of the southeastern flora. New York. 1933.
7. Smith, R. W., The tetranucleate embryo sac of *Clintonia*. Bot. Gaz. **52**:209–217. 1911.
8. Strasburger, E., Über Polyembryonie. Jenaische Zeitschr. **12**:647–670. 1878.

CYTOLOGICAL STUDIES IN RELATION TO THE CLASSIFICATION OF THE GENUS CALOCHORTUS. II[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 525

J. M. BEAL

(WITH THREE FIGURES)

In a previous paper (1) the number and morphology of the chromosomes of twenty-eight species and five varieties of *Calochortus* were reported. The taxonomic arrangement followed therein had been furnished by Dr. MARION OWNBEY, who has since published his monograph (2) of the genus, covering a total of fifty-seven species and thirteen varieties. Three additional species have since been investigated and their somatic chromosome numbers determined. They represent two of the three sections into which the genus is divided, and they conform in chromosome numbers with the other species previously investigated cytologically and assigned to these sections.

Bulbs of *C. lobbii* were secured from Mr. Carl Purdy, Ukiah, California, and those of *C. weedii* var. *vestus* and *C. barbatus* were kindly supplied by Mr. William M. James, Santa Barbara, California. *C. weedii* var. *vestus* had been collected locally, while *C. barbatus* had been secured by Mr. James from Mr. George B. Hinton, Mina Rincon via Toluca, Mexico. The bulbs were potted in a sandy loam soil and kept in a cool room in the greenhouse until roots began to emerge. Fixation was made in La Cour's 2 BE solution and the tips were imbedded in paraffin according to the usual schedule. Transverse sections were cut at 20–25μ and stained with the gentian violet iodine stain.

The three species reported here are assigned to the sections and subsections in conformity with OWNBEY'S monograph (2).

SECTION I. EUCALOCHORTUS

Subsection 2. Eleganti

C. lobbii Purdy.—Diploid, 20 chromosomes (fig. 1). The number agrees with and the morphology of the chromosomes conforms in general with those of other species investigated in the section.

[1] This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

Section III. Cyclobothra

Subsection 9. Weediani

C. weedii var. *vestus* Purdy.—Diploid, 18 chromosomes (fig. 2). This species agrees in number with that previously determined for *C. plummerae* Greene, a member of the same subsection, but the chromosomes appear somewhat thicker, a condition which may perhaps be caused by the fixing solution.

C. barbatus (HBK) Painter.—Diploid, 36 chromosomes (fig. 3). This species is apparently tetraploid since at least certain of its chromosomes occur in quadruplicate.

Figs. 1–3.—Root tip metaphases: fig. 1, *C. lobbii*, $2n = 20$; fig. 2, *C. weedii* var. *vestus*, $2n = 18$; fig. 3 *C. barbatus*, $2n = 36$.

The number of species of the Cyclobothra section thus far investigated is three, and all of them show 9 as the basic number of chromosomes. It would be of interest if still other species were to be studied to determine whether this basic number may be constant for the section.

An interesting feature of the cytological studies of *Calochortus* has been the establishment of the close correlation between the number and morphology of the chromosomes on the one hand and the morphologic and organographic characters on the other, both of which were used by Ownbey as criteria for the separation of the genus into sections and subsections and the assignment of species under them.

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LITERATURE CITED

1. Beal, J. M., Cytological studies in relation to the classification of the genus *Calochortus*. Bot. Gaz. **100**:528–547. 1939.
2. Ownbey, Marion, A monograph of the genus *Calochortus*. Ann. Missouri Bot. Gard. **27**: 371–560. 1940.

WATER CONTENT AND OSMOTIC PRESSURE OF SUN AND SHADE LEAVES OF CERTAIN WOODY PRAIRIE PLANTS

FRANK L. MARSH

A recent study of the water content and osmotic pressure of certain herbaceous prairie plants in the vicinity of Lincoln, Nebraska, emphasized the influence of direct sunlight on the development of relatively high osmotic pressures (2). A similar effect was observed in woody prairie plants of the same locality. These previously unpublished data include results of experiments with sun and shade leaves of *Cornus stolonifera*, *Rhus glabra*, *Sambucus canadensis*, and *Vitis vulpina*.

Material and methods

Leaves in sun and shade were collected from 1:00 to 2:00 P.M. on sunny days and immediately placed on solid carbon dioxide in small screw-top aluminum cans, where they were frozen crisp in a few minutes. The material was thawed the following morning and the sap expressed immediately under a pressure of 10,000 pounds per square inch. The depression of the freezing point was promptly obtained by an ether-evaporation device (2). Osmotic pressures were found by using the corrected tables of HARRIS (1).

Leaves for water-content determination were secured in amounts of 75 gm. in small, screw-top glass jars. This material was a duplicate of that used in obtaining osmotic pressures and was collected at the same time. Samples were dried at 80° C. for at least 48 hours. Calculations were based on green weight.

Material was obtained from plants growing in a shallow ravine where soil moisture was abundant. Only mature, fully expanded leaves were used. Sun leaves were collected on the south side at the periphery near the top of the plant. Under the intense sunlight of this region, these leaves were definitely thicker and smaller than those situated in the shade only a few inches in from the periphery; planimeter measurements of the areas of upper surfaces of sun leaves of *Cornus* gave an average of 2.7 square inches while the area for shade leaves was 6.4.

The shade leaves were collected at a point just beneath the peripheral canopy. A steady south wind blew at the time, so that the atmosphere surrounding the leaves in both situations had the same relative humidity. Psychrometer readings were the same at the periphery as just beneath it, since such readings are always taken with the wet and dry bulbs shaded. However, the actual temperatures of sun leaves *in situ* were 5–7° F. higher than were those of the shade leaves (table 1).

These temperatures were obtained by folding the illuminated or shaded leaves closely over the thermometer bulb, the resultant readings indicating the internal temperatures of the leaves. The external physical factors of these sun and shade leaves were identical except for the direct sunlight upon the former and diffuse sunlight upon the latter, with accompanying temperature differences.

Results

Sun leaves of the four species studied had 1.1–11.6 per cent lower water content than the shade leaves (table 1). Osmotic pressures of the former were 2.1–5.4 atmospheres higher than those of the latter.

TABLE 1

OSMOTIC PRESSURE AND WATER CONTENT OF MATURE SUN AND SHADE LEAVES IN VICINITY OF LINCOLN, NEBRASKA

Plant	Height (inches)	Leaf	Light (foot candles)	Temperature (°F.)	Water content (%)	Osmotic pressure (atmospheres)
Cornus stolonifera	60	Shade	175	83	72.2	10.5
		Sun	10,000	89	60.6	15.9
Rhus glabra	60	Shade	400	84	66.1	10.7
		Sun	10,000	89	65.0	12.8
Sambucus canadensis	60	Shade	100	82	84.9	8.8
		Sun	10,000	89	75.8	12.3
Vitis vulpina	60	Shade	40	82	76.5	6.4
		Sun	10,000	88	71.8	9.2

Discussion

It has been shown that where soil moisture is abundant there may be no correlation between water content and osmotic pressure of the tissues of herbaceous prairie plants (2). In such situations, water content may be highest in the same portion of the stem in which there is the greatest amount of solutes. Likewise, in leaves of plants which have abundance of available soil moisture, osmotic pressure may increase at the same time that water content is increasing. As shown in this study, however, sun leaves had higher osmotic pressures and lower water content than shade leaves, regardless of abundance of water at the roots. The higher osmotic pressure of the former was apparently due to the lower water content of the tissues.

In all cases the shade leaves were sufficiently illuminated to produce maximum photosynthesis, but the total amount of infra-red rays was much less than that falling upon the sun leaves. This latter fact is evidenced by the lower temperatures

of the shade leaves. According to SPOEHR (**4**), about 50 per cent of the total radiant energy of the sun is transformed in the leaf and used in the vaporization of water. It is well known that transpiration in sun leaves is more rapid than in shade leaves. In some plants a linear relation between water loss and light intensity has been determined under a rather wide range of conditions (**3**). It appears correct to assume that, under the external physical factors of this experiment, photosynthate was as abundant in the shade leaves as in the sun leaves. However, the higher rate of water evaporation from the latter produced a greater concentration of osmotically active substances.

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LITERATURE CITED

1. HARRIS, J. A., An extension to 5.99° of tables to determine the osmotic pressure of expressed vegetable saps from the depression of the freezing point. Amer. Jour. Bot. **2**:418–419. 1915.
2. MARSH, F. L., Water content and osmotic pressure of certain prairie plants in relation to environment. Univ. of Nebraska Studies, Vol. XL, no. 3. 1940.
3. MARTIN, E. V., Effect of solar radiation on transpiration of *Helianthus annuus*. Plant Physiol. **10**:341–354. 1935.
4. SPOEHR, H. A., Photosynthesis. Chemical Catalog Co., New York. 1926.

METHOD FOR SOFTENING FILBERT BUDS IMBEDDED IN PARAFFIN

C. E. SCHUSTER[1]

Repeated attempts to section filbert buds and filbert pistils have been made by the writer since 1920. Even dissecting out the pistils produced little success, owing to the inability to cut the hairs at the base of the pistils. Since 1935 an intensive study has been carried on, during which time practically all reported methods and materials for such type of work were tried out, as well as many suggested by co-workers. Some improvement over standard methods was made, but not to the degree desirable. The material collected during January-April consistently resisted successful sectioning.

Of the methods and materials tried, soaking the paraffin-imbedded buds in water was best, but even that method failed to give results desired. Too many sections were torn across by the knife, and from some samples no sections were made that could be studied satisfactorily. Blocks set aside in water and forgotten until the blocks were slimy and the water thick with molds and slime gave the clue to part of the methods reported here. No attempt has been made as yet to identify the product or organisms responsible for the action on the imbedded plant material, but trials will be undertaken soon to study and isolate the organisms.

The method as worked out is the result of attempts to section filbert pistils for gross studies of pollen tubes, tube cysts, and similar developments in the pistil. It might not be suited for material intended for study of finer details.

The procedure as finally revised is as follows:

1. Solution A

   10 cc. 2% chromic acid (water solution)
   100 cc. 95% ethyl alcohol
   100 cc. 36% glacial acetic acid

   Solution B

   40 cc. 40% formalin
   100 cc. 95% ethyl alcohol

   Use equal parts of solutions A and B. Unless whole buds are needed for study, dissect out the parts desired, as it is difficult to obtain a good infiltration of paraffin. The material should remain in the killing and fixing agent for at least 24 hours.

[1] Horticulturist, Bureau of Plant Industry, United States Department of Agriculture.

The writer appreciates the assistance of EDNA GEIL and WILLIAM EVENDEN, Laboratory Assistants, in this work. Acknowledgments are made also to Dr. W. B. BOLLEN, Associate Bacteriologist, Oregon Experiment Station, for help and suggestions. Assistance was furnished by the Works Progress Administration under projects W.P. 1656, O.P. 465-94-3-39 and W.P. 2573, O.P. 665-94-3-38.

2. Boil in 50% dioxan for 40 minutes.

Place vials containing buds in a water bath under a ventilating hood. (Dioxan fumes are extremely injurious to health.) After boiling, pour off the dioxan.

3. Use zinc chloride, 10 gm. in 33.6 cc. of 35% hydrochloric acid, diluted with water as noted below:
   a) 12½% zinc chloride and hydrochloric acid, 2 hours
   b) 25% zinc chloride and hydrochloric acid, 2 hours
   c) 50% zinc chloride and hydrochloric acid, 2 hours
   d) 75% zinc chloride and hydrochloric acid, 1 hour
   e) 100% zinc chloride and hydrochloric acid, ¼–½ hour
   Exact length of time determined by degree of hydrolysis attained or desired.

4. Dehydrate in dioxan.
   a) 25% dioxan (three changes), 2 hours
   b) 50% dioxan, 1 hour
   c) 75% dioxan, 1 hour
   d) 100% dioxan and butyl alcohol, equal parts, three changes, ½ hour each. Add one drop of oil to each cubic centimeter of solution in the last change.

The writer has used cellusolve according to HANCE.[2] It shortens the operations and time occupied in 2, 3, and 4, but the results in staining have not been so satisfactory as with the longer schedule. It also aids in softening tissue and for some tissues may be suitable, but with filbert buds, etc., softening did not progress to the point where satisfactory sections could be consistently cut.

5. Put in vials and fill with shaved paraffin, place on oven over night and in oven in the morning. Pour off and add pure paraffin, changing three or four times and allowing ½ hour for each change. Avoid keeping in the oven longer than necessary for good infiltration of the paraffin, as this process hardens the hairs.

6. Treatment of paraffin blocks:
   a) Cut imbedded material into small blocks ⅓–½ inch square and soak in water for several months. (For filberts the buds have been soaked for at least 6 months.)
   b) Remove from containers and place in open dishes containing a bacterial suspension, prepared by placing dead and decaying straw and grass in the dish with a solution of:
   ½ gm. ammonium nitrate
   1000 cc. water
   Fill small dishes with this stock solution and place more straw and paraffin blocks therein. Keep in a warm room out of the sunlight for 2 or more weeks, adding more stock solution as evaporation progresses.

It has been impossible to cut suitable sections simply by soaking in pure water for 6 months, nor has it been possible to obtain satisfactory sections with the bacterial suspension for a few weeks alone. The combination of the two treatments, however, has

[2] New histological methods. Gradwohl Laboratory Digest. Vol. II, no. 9. 1937.

given excellent results. Whole buds can be sectioned at 10 μ. Of the pistils and attached tissues, sections 5–7 μ thick are readily cut without tearing.

The paraffin blocks are chilled in an icebath and cooled from time to time with blocks of ice as cutting progresses. The microtome knife must be sharp, and it is prepared for use according to directions given by EVENDEN and SCHUSTER.[3]

The following stains have been used successfully on filbert buds sectioned by the preceding schedule. Apparently the treatment does not greatly affect staining qualities of filbert tissues.

a) Leaf buds.—Haematoxylin with one drop of tergitol no. 7 added to a jar of stain.

b) Pistillate buds through pollinating season.—Orseilline B.B. and light green combination.

c) Pistillate buds from beginning of embryonic tissue.—Orseilline B.B., fast green F.C.F., methyl violet 2B, and orange G, according to JOHANSEN,[4] except that orseilline B.B. is substituted for safranin O.

[3] A new sharpening back and procedure of sharpening for microtome knives. Stain Technol. **14**:69–71. 1939.

[4] A quadruple stain combination for plant tissues. Stain Technol. **14**:125–128. 1939.

CURRENT LITERATURE

Modern Fruit Production. By J. H. GOURLEY and F. S. HOWLETT. New York: Macmillan Co., 1941. Pp. vi+579.

This volume illustrates the great strides made in the last 30 years toward the merging of the abstract and the more closely applied aspects of plant science. The fields of ecology, physiology, and morphology are well represented in the chapters dealing with factors affecting flower formation, general cultural practices, water relations, fruit setting and thinning, nutrient deficiencies, the origin and improvement of fruits, etc.

The book is much more than a compilation. Many practices are analyzed in detail in the light of existent botanical knowledge, whereas others are less clearly defined. In this there seems to be no alternative possibility, for botanical investigation must be more penetrating far before anything approaching detailed analyses of field practices can be made. There might well be disagreement with some of the interpretations of the authors on the body of evidence considered; but it is their views which give distinctive character to the book.

The illustrations are relatively scarce, but in the main are informative and to the point. The reader will note the omission of much of the older standard literature, not a particularly serious loss, since—with some additions and modifications—many of the more recent contributions largely repeat and embody the same material. The entire book is pleasingly readable and thoroughly usable to the botanist, the horticulturist, and anyone seriously interested in fruit crops. It is a worthy addition to the slowly growing list of texts attempting a rationalization of agricultural practice in terms of progressive research.—E. J. KRAUS.

Photosynthesis. By E. C. C. BALY. New York: D. Van Nostrand Co., 1941. Pp. vi+248. Figs. 24. $4.75.

In this third monograph to bear this title (two by other authors having appeared in 1926), BALY presents in full the various contributions he has made to the problems of photochemical synthesis of carbohydrates and nitrogenous compounds in the last 20 years. The work is somewhat narrow in its scope, since no serious attempt has been made to incorporate into the discussion the extensive research done in this country. The work of EMERSON and SPOEHR is included, but no other important American contributions are cited.

The early chapters, introductory, deal with the difficulties of interpreting photosynthesis from the standpoint of photodynamics and quantum relationships; they also include a retrospective examination of the early investigations on the action of ultraviolet radiations on solutions of carbon dioxide, on the polymerization of formaldehyde into glucose, and on the photosynthesis of carbohydrates by the action of light on hydrated carbon dioxide adsorbed on a surface. Chapter V discusses the final achievement of photosynthesis of carbohydrates, and deals with the more recent investigations. A chapter follows on the assimilation of nitrogen by the living plant. The last two chapters attempt to picture the mechanism of photosynthesis and the kinetics of the process. The author index lists slightly over 100 names, and a subject index contains about three pages.

Well written, easy to read and understand, this volume's main value is to bring together and summarize the author's own contributions and viewpoints. Some of these have aroused controversial discussion as to the adequacy of the experimental techniques and as to the validity of the conclusions, which in some cases have seemed highly hypothetical. The work should be welcomed and enjoyed by those who, keeping this in mind, can read it with well-balanced critical perspective. It should stimulate a valuable rethinking of the problems and a better experimental approach to the final solution and acceptance of a mechanism in full harmony with the kinetic requirements.—C. A. SHULL.

Flowers and Flowering Plants. 2d ed. By RAYMOND J. POOL. New York: McGraw-Hill Book Co. Inc., 1941. Pp. xxiii+428. Figs. 211. Folded chart. $3.50.

The new material included in this edition was largely selected from suggestions made by teachers of plant taxonomy. A few minor rearrangements of the subject matter give the book greater coherence. A new chapter on the vegetative organs of plants, with particular emphasis on the terminology of leaf margins, tips, bases, etc., as well as the appended glossary, adds to the completeness of the text. Revision of the map indicating the useful ranges of plant manuals in the United States brings the reference matter up to date.

A noteworthy change is made in the folded chart facing page 150. The relative phylogenetic positions of the orders of flowering plants are shifted only slightly when compared with the chart of the first edition, but the families are omitted, except for some of the apetalous and anemophilous groups. A habit picture, a cross-sectional floral diagram, and a composite floral formula are given for each order named on the chart. Most teachers of systematic botany will applaud this simplification. Even though no adequate explanation of the method of applying scientific names to plants is given, this work is the most useful text in its field.—P. D. VOTH.

Biological Symposia. Vol. II. Edited by JAQUES CATTELL. Lancaster, Pennsylvania: Jaques Cattell Press, 1941. Pp. 270.

This second volume consists of some 21 papers which were presented at the Milwaukee meeting of the American Association for the Advancement of Science, June 20, 1939; the Columbus meeting, December 28, 1939; and the meeting of the Western Society of Naturalists at Monterey, December 20, 1939.

The subject matter is presented under four sections: (1) speciation; (2) defense mechanisms in plants and animals; (3) biological basis of social problems; and (4) regeneration. The bringing together in one volume of such a group of scholarly papers, thus making them available to all interested, is a valuable service to science. The editor merits the commendation of all biologists.—J. M. BEAL.

Hunger Signs in Crops. A Symposium. Washington, D.C.: Judd and Detweiler (published by American Society of Agronomy and National Fertilizer Association), 1941. Pp. xiii+327. Illustrated. $2.50.

A symposium on nutritional deficiency symptoms, presented in nine chapters, with fourteen individual contributors. Nearly 80 colored plates are used in illustrating the symptoms, and almost 100 uncolored figures. The first chapter is introductory, discussing why plants starve. The succeeding chapters take up specific crops, or closely related groups. The stories of starvation and response are told in simple, direct language. Literature references are not used. As the authors in every case are experts with the crops considered, the book is an authoritative

and accurate summary of nutrient deficiencies in our main agricultural crops. This work is an example of cooperation between science and industry, and because it is, it costs far less than if it had been produced wholly as a scientific venture. This book gives adequate information on a subject of widely growing popular interest, since correct plant nutrition is the basis of safe nutrition for animals and man. It is an outstanding contribution to popular understanding of an important field of plant physiology.—C. A. SHULL.

Bibliography of References to the Literature on the Minor Elements and Their Relation to Plant and Animal Nutrition. Second Supplement to Third Edition. New York: Chilean Nitrate Educational Bureau, Inc., 120 Broadway, 1941.

The second supplement to this important bibliography contains 67 pages; almost 500 references with brief abstracts; and the threefold index, by elements, botanical species, and authors. Almost 50 of the elements are listed, and approximately 150 species of plants—by their common names usually. The entire work is the most useful bibliography in the field of plant nutrition and constitutes an outstanding contribution to the research facilities of the plant physiologist. The Chilean Nitrate Educational Bureau deserves high commendation for continuing this vital service to plant science.—C. A. SHULL.

FIFTIETH ANNIVERSARY CELEBRATION

☼ ☼ ☼

In connection with the Fiftieth Anniversary Celebration of The University of Chicago, a Symposium on Growth and Differentiation in Plants will be held by the botanists, Monday morning, September 22, 1941.

Papers for the session will be contributed by

DR. C. E. ALLEN, University of Wisconsin
DR. J. M. BEAL, University of Chicago
DR. E. W. SINNOTT, Yale University
DR. J. W. MITCHELL, U.S. Department of Agriculture

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